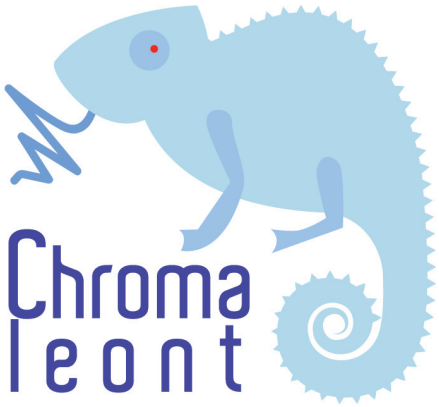

Chrom^{square} 2.3 User Manual

GCxGC/MS & LCxGC/MS version for Shimadzu GCMSsolution and GCsolution

Chromaleont srl

December 2, 2015 - Release 2.3

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Foreword

1.1 Contact Information

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1.2 Legal Information

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Any error or omission which may have occurred in this publication will be corrected as soon as possible, but not necessary immediately upon detection.

Chromaleont makes no claims or warranty concerning the influence of the application of this publication on the operation of the machine.

Note that Chromaleont does not have any obligation concerning the effects resulting from the application of the contents of this manual.

1.2.2 Warranty

Chromaleont provides for this product the warranty described in the following paragraphs.

1.2.2.1 Period

Please contact Chromaleont at chromaleont@chromaleont.it for information about the period of this warranty.

1.2.2.2 Description

If a product/part failure occurs for reasons attributable to Chromaleont during the warranty period, Chromaleont will repair or replace the product/part free of charge. However, in the case of products which are usually available on the market only for a short time, such as personal computers and their peripherals/parts, Chromaleont may not be able to provide identical replacement products.

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Failures caused by the following are excluded from the warranty, even if they occur during the warranty period.

1. Improper product handling.
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3. Product use in combination with hardware or software other than that designated by Chromaleont.
4. Computer viruses leading to device failures and damage to data and software, including the product's basic software.
5. Power failures leading to device failures and damage to data and software, including the product's basic software.
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7. Reasons unrelated to the product itself.
8. Product use in harsh environments, such as those subject to high temperatures or humidity levels, corrosive gases, or strong vibrations.
9. Fires, earthquakes, or any other act of nature, contamination by radioactive or hazardous substances, or any other force major event, including wars, riots, and crimes.
10. Product movement or transportation after installation.
11. Consumable items (Recording media such as floppy disks and CD-ROMs are included).

1.3 Introduction

1.3.1 Outline of the Software

Congratulations on your purchase of the Chromaleont *Chrom*^{square} GCxGC/MS & LCxGC/MS version for Shimadzu GCMSsolution and GCsolution.

Chrom^{square} workstation software is designed for *visualizing*, *processing*, and *reporting* on data obtained by *Two-Dimensional Chromatography*.

A license and USB Bluetooth Key is required for execution. A trial version, with 60 days soft-key license, is also available at no charge.

The software and the documentation are released with *different license types*, listed in the next Section. License types refer to the types of data files that the software can analyze, namely:

- data files in proprietary format, generated by various kinds of Shimadzu instruments;
- data files in open format (AIA/ANDI).

There is also a general purpose license type which includes all the other ones.

1.3.1.1 License types

Presently the following license types are provided:

1. GCxGC/MS & LCxGC/MS version for Shimadzu GCMSsolution and GCsolution
2. LCxLC version for Shimadzu LabSolutions LCMS
3. LCxLC version for Shimadzu LCMSsolution and LabSolutions LCMS
4. Text and NetCDF Version
5. Text and NetCDF Version with NIST Spectra Search
6. All Features Version

The present Manual is specific for GCxGC/MS & LCxGC/MS version for Shimadzu GCMSsolution and GCsolution license type. Manuals relative to other licenses may be furnished on demand.

1.3.2 Outline of the Manual

1.3.2.1 Prerequisites

Please read this Instruction Manual carefully before using the product and operate the product in accordance with the instruction given thereby. Keep this instruction manual for future reference.

This Instruction material was written with the assumption that the reader has some knowledge of MS-Windows. For information on the names and terminology associated with MS-Windows, please refer to the MS-Windows user documentation. If you are using MS-Windows for the first time, please read the MS-Windows user documentation prior to reading this Help material.

1.3.2.2 Structure

The Manual can be considered as consisting of *four parts*:

1. the first part is a *general introduction* and includes the Chapters 1, 2.1.2.1, 3;
2. the second part is an *analytical description* of all parts of the Graphical User Interface (the Menu and the three Main Panels); it includes Chapters 4, 5, 6, 7;

3. the third part is a detailed description of the main *routine operations*: namely, Qualitative Qualitative Analysis, Quantitative Analysis, Statistics; it includes Chapters 8, 9, 10;

4. the fourth part is a description of various *advanced operations*; it includes Chapter 11 and 12.

1.3.2.3 How to use this Manual

We recommend that the user carefully read the following "key" chapters:

- Chapter 3 (Overview): this gives an essential introduction to the main concepts and terminology;
- Chapter 8 (Qualitative Analysis): this describes step by step the most common usage of the software;
- Chapter 9 (Quantitative Analysis): the same as before, for users which will use the software for quantitation.

All others chapters may be used as *reference*: that is, they do not need to be read sequentially, but more profitably consulted upon the frequent references from the aforementioned key Chapters.

1.3.2.4 How to understand the Figures

The Figures reproduce screen shots (or parts of them) taken during software operations.

Figures have the main function of supporting the user to better interpret the contents of the written text, and to avoid misunderstandings about the use of the graphical objects.

They do not always represent an exact reproduction of software behaviour, since this is affected by to many factors (like license types and choice of sample data files). In same cases, where the type of license makes the difference, the Figures refers to that very license; in other cases, when the Figure is just used to explain an operation not depending on any particular license, the Figure may contain references to a license different from that to which the manual refers.

1.3.3 What's new in *Chrom*^{square} 2.0

Chrom^{square} 2.0 represents a major improvement on the previous versions of the software: it contains several enhancements, described in various parts of the present manual.

The users who are already familiar with *Chrom*^{square} can find in this section a list of the new features, with links to the sections where they are described.

- New arrangement of Views in the Chromatogram Panel (Section 6.1).
- New context menu in Map View (Section 6.2.5).
- Customization of Data Analysis Table (Section 6.5.2).
- Method Panel parameters section entirely redesigned (Section 7.2).
- Enhanced Spectra search (Section 7.2.3).
- Introduction of *Levels* in the Method (Section 7.2.5.2).
- New Tool for Statistics calculation (Section 10).
- New Report Template Editor, fully redesigned (Section 11.5).
- New Blob Edit tool (Section 12.1).

1.3.4 What's new in *Chrom*^{square} 2.2

Chrom^{square} 2.3 includes the following main enhancements:

- *Exact mass* handling: a new option for assigning the number of decimal digits of m/z both in the Spectrum View and in the Spectra Search (Section 7.4).
- *UV Spectra* handling (Section 6.2.5.10 and following ones, 6.7.2, 7.5).
- *Enabling and disabling synchronizations* between Map View ond other views (Section 4.5.3.8)

1.3.5 What's new in $Chrom^{square}$ 2.3

$Chrom^{square}$ 2.3 includes the following main enhancements:

- Possibility of loading *all Mass Spectra events* stored in a Shimadzu data file.
- *Advanced Ion Selection* (multiple SCAN, SIM, MRM events) defined though a graphical editor.
- *Advanced MRM spectra management*, both for qualitative and quantitative analysis, and relative graphical editor.
- New software protection and licensing, based on the *WIBU key* technology.

Chapter 2

Installation and Startup

2.1 Prerequisites to install *Chrom*^{square} with Shimadzu software

2.1.1 Interface with Shimadzu GCMSsolution software

2.1.1.1 Compatibility

Since *Chrom*^{square} version 1.5 the spectra search function is deeply integrated with Shimadzu GCMSsolution.

An agreement with Shimadzu Co. allows to perform a seamless search using `GCMSsolution 4.11` or higher.

The spectra search function will not work with previous versions of GCMSsolution; *datafiles can be read anyway.*

2.1.2 Interface with Shimadzu LCMSsolution and LabSolutions software

2.1.2.1 Compatibility

All *Chrom*^{square} 2.3 software functions described in the present manual that involve interactions with LC Shimadzu software have been developed and tested on systems where were installed one of the following Shimadzu softwares and relative upgrades:

- `Shimadzu LabSolutions Version 5.72`, with `TTFLDataExport Ver 5` Upgrade.
- `Shimadzu LCMSSolutions Version 4.03`, with `TTFLDataExport Ver 3` Upgrade.

Chrom^{square} 2.3 may work also with some previous versions of the Shimadzu software, provided that the last `TTFLDataExport` patch has been installed.

Please contact Shimadzu Co. for a detailed list of compatibilities and for downloading the last upgrades.

2.2 Installing the software

The *Chrom*^{square} is a *Java application*: this means that it may run under *any operating system*, provided that a suitable *Java Virtual Machine* is installed on it[1].

Before installing the *Chrom*^{square} software verify that a recent *Java Runtime Environment* (version 1.7 or later) is installed. For free download, visit the site `http://www.java.com`.

The install disk is provided as a CD-ROM. To install the *Chrom*^{square} software, insert the CD-ROM into the driver. The disk contains a bootstrap installation.

The installation procedure is a very straightforward, very similar to the most common installation procedures. The user should navigate among various dialog boxes, answering to very simple questions.

In the following steps, an outline of the installation procedure is reported. The various windows make a sequence that may be navigated in both directions: at any time it is possible to go back to correct some parameter, but normally the user advances through a series of `Next` commands.

1. The first window is a *Prerequisite* window: it warns the user about some hardware requirements. If the requirements are satisfied, select `Yes` to continue with the installation.
2. The next window is a *Welcome* window; no answer is required; select `Next` to continue.

[1]The compatibility does not apply to some components which provide the interaction with the instrumentation software, which are only compliant with Microsoft Windows systems.

3. The next window informs asks the user for he size of the Java Virtual Machine: a default size of 1024 KB (1 GB) is suggested. A smaller value may be specified for computers having less then 2 GB of memory, in order to leave enough space for other applications[2]; a bigger value may be provided for more capable computers, remembering however that 2048 KB is the maximum allowed size on a 32 bit system. Select `Next` to continue.

4. The next windows is *Select Destination Location*. The software suggests as default the folder `Program Files\ChromSquare`[3]. Select `Next` to continue.

5. The next windows is *Select Start Menu Folder*. The software suggests as default the folder ChromSquare. It is recommended not to change the default value. Select `Next` to continue.

6. The next windows is *Select Additional Tasks*. The software asks for creating a *desktop icon*. It is recommended *to check the box*. Select `Next` to continue.

   Note. The installation procedure creates a second ChromSquare icon, labeled *Debug Mode*, in the Program Group (this second icon is not displayed on the desktop); the icon allows to run the application displaying an informative window, called *Java Console* (see See Section 3.4). The Java Console may be useful to detect errors; it does not affect the software performance (only in some cases it can run a little slowly).

   Normally the user should not use this second icon, except when requested from the Technical Assistance; anyway, running in Debug Mode does not do any harm, and the Java Console can be closed at any moment.

7. The next windows is *Ready to Install*. This is a final review of the previous steps, before proceeding with the very installation. Select `Install` to continue.

8. The *Installing* window is then displayed, showing a progress bar which lasts for some instants; at the end the window will close automatically.

9. The next window is *Check the Program Files*: this is an integrity check procedure, that will check that all important files have been correctly downloaded by the installation CD. Press the button `Execute` and wait a few moments. At the end, the integrity check result is shown (Passed or Failed):

[2]This is very important for the cases which require the interaction of $Chrom^{square}$ with third party software.

[3]In former $Chrom^{square}$ versions the suggested folder was at disk root level, namely C:\ChromSquare. This has been changed in order to comply the common Windows standards. Anyway, the user can still choose the old location.

in the case that the check fails, please generate a detailed report with the button `Notepad`, and send it to the software distributor in order to get a new CD. Press the button `Cancel` to close the procedure and continue with the installation.

10. The installation procedure terminates asking the user for some optional installations.

    - The first one is about the *Visual C++ 2013 Redistributable Package*. This is a set of runtime Microsoft components that are necessary to interface with Shimadzu instrumentation software. The package should be installed just one time, so if you are upgrading an existing installation it is not necessary to install it again (in this case you may answer *No*; anyway, answering *Yes* the package will be reinstalled without any harm). In some case, the package will not be installed since a more recent version of the package had already been installed on the computer: in this case a warning message will be shown, and the user can continue the installation without additional operations.
    - The second optional installation concerns the installation of a set of *sample files*; sample files are specific for the license purchased. Answer `Yes` if this is the first installation or an upgrade of a previous installation: sample files are very useful for testing the software before real use and they do not require too much disk space; answer `No` if you are just re-installing the software starting from the same media; in case of doubt, answer `Yes`.

      The installation of sample files is accomplished through a Command Prompt window, that appears and disappears in few seconds, except in the case that other sample files are found: in this case the Command Prompt window will ask if you want to overwrite existing sample files; even in this case, answer `Yes`.

      At the end, a message will inform the user about the folder used for the sample files installation.
    - The third optional installation concerns the *manual files*; manual files are specific for the license purchased. Answer `Yes` if this is the first installation or an upgrade of the software: manual files may change between different releases of the software; answer `No` only if you are re-installing the software starting from the same media; in case of doubt, answer `Yes`.

      The installation of manual files is accomplished through a Command Prompt window, that appears and disappears in few seconds, except in

the case that other manual files are found: in this case the Command Prompt window will ask if you want to overwrite existing manual files; even in this case, answer `Yes`.

At the end, a message will inform the user about the folder used for the manual files installation.

Note: the windows about the optional installations are shown only if the installation package contains the samples or manual files; if you are installing an upgrade of the software, it is possible that these files are not present, and the corresponding windows are not shown.

11. The last window (*Completing the Chrom^{square} Setup Wizard*) terminates the installation procedure. If you want to launch immediately the program, tick the appropriate check box and then the `Finish` button.

2.3 Uninstalling the software

There is no specific uninstall procedure of the software: just use the standard Windows uninstall facility, as follows:

- From the Windows `Start` menu, choose `Settings`;
- then click `Control Panel`;
- double click the `Add/Remove Programs` icon;
- select `ChromSquare`;
- choose `Remove`.

The procedure may slightly vary according to the different Windows versions and languages.

2.4 Starting *Chrom^{square}* software

To execute *Chrom^{square}*, use the *desktop icon* or the corresponding item of the Start menu (see Figure 2.1).

A *splash screen* containing information about the software and its producer is shown during the few seconds required to load the application software.

Figure 2.1: The *Chrom*^{square} 2.3 desktop icon

2.5 License

2.5.1 The WIBU protection system

Chrom^{square} software always requires a *license*: this may be a *regular license* or a *demo license*.

Both regular and demo licenses enable the software to handle one or more types of data files. A special type ("All purposes") enables all types of data files.

Starting from Release 2.3 all licenses are based on an hardware component called "WIBU" key: this is a special USB key, produced by WIBU Systems AG, a leader corporation in the field of software protection.

A WIBU key contains all license information. Key and license *are bounded to the user, not to the computer*: it is then possible to run the software on more computers with a single license, by simply moving the WIBU key from one computer to another. The software regularly checks if the key is still connected, and interrupts the execution if it is not found.

2.5.2 Temporary Licenses vs. Regular licenses

A WIBU key is always required in order to run the software; a WIBU key with a temporary license may be provided for software evaluation; temporary licenses can be renewed or upgraded to regular licenses by means of simple procedures that do not require to move or exchange the physical WIBU USB device.

Chapter 3

Chrom^{square} Overview

3.1 General Terminology and Concepts

This chapter introduces some terms thoroughly used by the software and in the manual text. Even if these terms should be already familiar to the software users in the field of bi-dimensional chromatography, it is important to specify exactly their meaning, since there are some subtle differences that must be cleared, to avoid misunderstanding of some descriptions.

3.1.1 Chromatogram

With this term we mean the data coming from chromatographic instrumentation; since data are normally recorded on files, the term *chromatogram* is used both for data loaded in memory and for files containing these data (more accurately, one should speak of *chromatographic data* and *chromatographic files*: for brevity, often we simply speak of *chromatograms*).

Chromatogram is basically a sequence of data, each datum being a couple of numeric values, corresponding to time and absorbance.

In bi-dimensional chromatography, chromatograms are acquired through a couple of instruments; the resulting data are normally recorded on a unique chromatogram. The overall chromatogram is then split into many slices, called *modulations*, according to a parameter called *modulation period* (in some case the original chromatogram corresponds to a set of files, each file corresponding to a single modulation: in these case, the overall chromatogram is build by the software joining together the single modulations).

In bi-dimensional chromatography, chromatograms are represented as bi-dimensional Maps.

3.1.2 Chromatographic Map

A Chromatographic Map is a bi-dimensional representation of a chromatogram, according to the following criteria:

- Horizontal Axis is a counter which represents the modulation number; this is equivalent to a time axis which measures the total time;
- Vertical Axis is a time axis which measures the modulation time, that is the time elapsed from the beginning of each modulation;
- Colors are used to represent the value of the absorbance at a given point; the relation between Colors and absorbance is ruled by a Color map, that can be configured by the user.

The Chromatographic Map is defined on a rectangular mesh of discrete points.

If the points are too much separated (this is frequent, especially in zoomed visualization) the graphic result is a set colored rectangles, resembling a pixel map too much enlarged. For a better visualization it is possible to perform an interpolation, that calculates a color value at each pixel point of the screen (and not only at the points of the mesh); in this way the result is a "smoothed" continuous map of colors.

3.1.3 Integration

Integration is the calculation process which analyzes the Chromatogram in order to recognize and identify Peaks (see 3.1.4) and Blobs (see 3.1.5):

- *Recognize* means to find the Blobs contours on the map. *Recognition* is geometrical analysis of the chromatogram map, that can be tuned on the basis of some parameters.
- *Identify* means to assign an identity (that is a *name*) to a recognized Blob. *Identification* is a process that is generally carried on manually by the user, which selects the Blobs with the mouse on the map and assign them a name; GCMSSolution license allows to perform an automatic identification; automatic identification is executed through calls to GCMSSolution software.

3.1.4 Peaks

A ***Peak*** is the basic object of any kind of chromatography: peaks are a significant variation of the absorbance signal along modulation time; we have a Peak when it is possible to recognize a maximum point (Peak Top), a Beginning Point, and an End Point.

3.1.5 Blobs

A ***Blob*** is a close geometrical figure, obtained by joining together the beginning and the end points of the same Peak in adjacent modulations.

Due to the discrete nature of Chromatogram data, Blobs figures are close polygons.

In $Chrom^{square}$ Blobs are data structures which contain various information, both geometrical and analytical.

3.1.5.1 Blobs: geometrical data

Geometrical data are defined when the Blob is recognized. They include:

- The description of the *Top* of the Blob (that is the point of the Blob having the *maximum intensity*; it corresponds to maximum of all Tops of the Peaks included in the Blob). The Top Blob is identified by:
  - the *Top Modulation Index* (that is the counter of the modulation corresponding to the Top);
  - the *Top Time Index* (that is the counter of the chromatographic point corresponding to the Top);
  - the *Total Retention Time* (also called `TtR`), that is the retention time of the chromatographic point corresponding to the Top;
  - the *Second Retention Time* (also Called `2tR`), that is the retention time of the chromatographic point corresponding to the Top measured along the *modulation*, i.e, the *second dimension*;
  - the *Blob Index*: this is an integer number which unequivocally identify the Blob, through a special combination of the Top Modulation Index and Top Time Index; the Index is useful in the various calculations, since it avoids the uncertainty arising from the approximations of decimal numbers.

- The description of the limits of the Blob in the two directions:
  - the *First Modulation* and the *Last Modulation*: two integer numbers which give the extent of the Blob along the First Dimension;
  - the *Top Modulation Start* and the *Top Modulation End*: two integer numbers which give the extent of the Blob along the Second Dimension (that is the first and last chromatographic points of the Blob measured along the modulation passing for the Top).
- The *Area* of the Blob, that is the *total area of all peaks which constitute the Blob*.

3.1.5.2 Blobs: analytical data

Analytical data are computed, and stored in the Blob data structure, as the various calculation steps are executed.

- After the completion of the recognition of all Blobs (*Integration*), the software can compute the *Area percent* of any single Blob.
- *During the identification* process, a *Blob ID* (see next Section) may be assigned to the Blobs (some Blobs could be remain unidentified and classified as "unknown"). To each identified Blobs, an *ID* and a *Name* are assigned.
- At the *end of the identification* process, the *Blobs Grouping* becomes possible: all Blobs which share the same Blob ID can be treated as single entities, named Groups. A series of new values (sum of the areas, other percentages with various normalizations) can now be computed.
- After the *Quantitative Analysis*, concentrations can be computed and stored in the Blob data structure.
- In the case of Spectral Search with LRI computation, the relative data are stored in the Blob data structure as well.

See the section 6.5.1 for other details and samples of the Blob data structure,

3.1.6 Blob IDs

A *Blob ID* is the *identification* of a Blob or of a group of adjacent Blobs. Assigning a Blob ID means to assign a *name* to a *region* of the chromatogram map. This could be achieved in two ways:

- *Manual Identification*: this is performed by the user, that draws with the mouse a rectangular or polygonal region around the Blob.
- *Automatic Identification*: this possibility is provided for chromatograms containing *Mass Spectra*: $Chrom^{square}$ can send spectral information about the region of the Blob to another software (Shimadzu software or NIST software) capable of spectral analysis and identification, based on its own or third party libraries.

Blob ID is a data structure containing various elements:

- The ***Name***, that is name of the chemical compound(s) corresponding to the Blob; note that names may be very long character strings.
- The ***ID***, that is an integer number which identifies the compound: a sort of abbreviation used for easier reference when more chromatograms are compared among them (like in the calibration process). The IDs are assigned in different ways:
  - for Blobs defined *manually*, the ID is just a *sequence number*, that is a *positive integer number* assigned by the software as the user defines new Blobs;
  - for Blobs defined *automatically*, the ID is a *negative integer number*, which is a sort of *fingerprint* (technically a *checksum*) of the name, calculated through a special algorithm named *CRC32*[1].
- The ***Region***, that is the geometrical description of the polygonal area. This information is provided for manually identified Blobs only.
- The ***Spectrum Data***, that is a full description of the Mass Spectrum used in the case of automatic identification. This information is obviously provided for automatic identified Blobs only.

The user can directly set and modify the first two items (IDs and names) through the Method Panel (see Section 7.2.5.1). Regions are set through a graphical procedure, described in Section 6.2.4.2 and 11.4.

[1] Whereas names may have very different lengths, the checksum are 32 bits negative numbers, ranging from -1 to about -4 billions (exactly -4,294,967,295); different names *almost* always generates different checksums; there is indeed a very small probability that different names may yield to the same checksum, but we can assume negligible this probability for our goals.

3.1.7 Blobs vs. Blob IDs

It is important to highlight the difference between *Blob* and *Blob ID*:

- Blobs are single objects, belonging to a chromatogram; Blobs are stored in the *Chrom^{square} Result File* (see 3.3.2).
- Blob IDs are analytical information, stored in a *Method*.
- In the first instance, *Integration* finds all the Blobs of a chromatogram; Blob IDs are unknown at this time.
- In the second instance, a Method (containing Blob IDs description) can be applied; after this step, some Blobs are *identified*, and a *Blob ID* is assigned to them.

3.1.8 Methods

A Method is a set of information that are used by the integration process.

A Method contains three kinds of information:

- *parameters*, that are used for Blobs recognition (see 7.2.2, 7.2.3, 7.2.4 for details);
- a list of *Blob identifiers* (called also *Blob IDs*, see 3.1.6);
- an optional list of *Groups*;
- an optional *Region of Interest* (also called ROI);
- an optional list of *Ions*.

Methods can be saved and loaded to/from special files, called *Method Files* (see 3.3.4).

3.1.9 Units

Time is always internally represented in *minutes*.

Tables and graphs generally adopt the usual conventions of chromatographic practice: that is, *minutes* for the *abscissa* axis (total retention time), *seconds* for the ordinate axis (modulations).

Absorbance is normally represented in *microVolts*, except in the particular cases, where absorbance is represented in *milliVolts*. Anyway please refer to the manual of the software that generated the data file that has been imported, since *Chrom*^{square} uses the same unit as it is defined in the original file.

3.2 *Chrom*^{square} Graphical User Interface

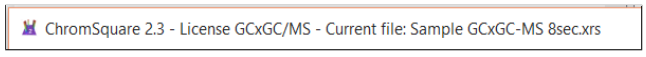
The *Chrom*^{square} Graphical User Interface consists of a Main Window and many secondary windows. It is important to know the exact names of these windows, in order to easily understand the description reported in the manual.

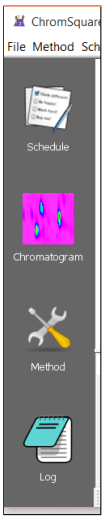
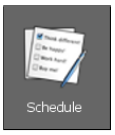
`In this manual, the windows names and other objects of the Graphical User Interface (menu items, toolbar icons, etc.) are represented with a monospaced font, that is the font used for the present paragraph.`

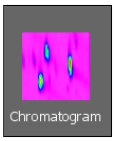
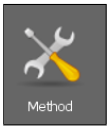
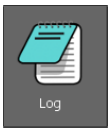
The *Chrom*^{square} Main Window is represented in Fig. 3.1. It includes - together with the usual Windows tool bars, like the Menu Bar and the upper Tool Bar - an important feature, called *Chrom*^{square} *Side Bar*, placed in the left side of the main window. This bar contains some large icons (presently 4, but others could be added in future releases); each icon behaves as a button which lets the user to switch among different panels: the `Schedule Panel`, the `Chromatogram Panel`, the `Method Panel`, the `Log Panel`.

In other words, the contents of the main window changes according to the Panel selected: in each moment the user can easily shift from one panel to another, without opening and closing specific windows.

The following table summarizes the list of items reported in Fig. 3.1.

Name	**Function**
Title Bar	 The Title Bar displays the program name, the license type, and the name of the chromatogram file currently loaded.

Menu Bar	 The Menu Bar represents the main access to all commands and options of the software: it contains all the operations for loading and saving files and workspaces, editing reports, viewing and saving images, switch on and off various graphical features and interpolation options.
Tool Bar	 Frequently used commands are assigned to the buttons of the Tool Bar, making their use easier. For a detailed explanation of the Tool bar, see the chapters describing the corresponding commands.
Side Bar	 Contains icons for switching among the main Panels.
Schedule Icon	 This icon is used to display the `Schedule Panel` for quantitative analysis.

Chromatogram Panel	 This icon is used to display the `Chromatogram Panel`, which represents the raw chromatogram as entire or as modulation chromatogram of a single fraction. In this window it is also possible to display the integration results, including retention times, compound names and quantitative results subsequent to data analysis.
Method Panel	 This icon is used to display the `Method Panel`, which lets to assign the Method parameters.
Log Panel	 This icon is used to display the `Log Panel`, which displays informative and error messages.

3.3 File Management

3.3.1 Raw Data Files vs. *Chrom*^{square} Files

Before to proceed with the description of the software operations, it is important to understand the File Management engine adopted by *Chrom*^{square}.

The File Management is based on the following concepts:

- The software reads *Raw Data Files*, that is files that are generated by instruments; these files may have different extensions, according to the kind

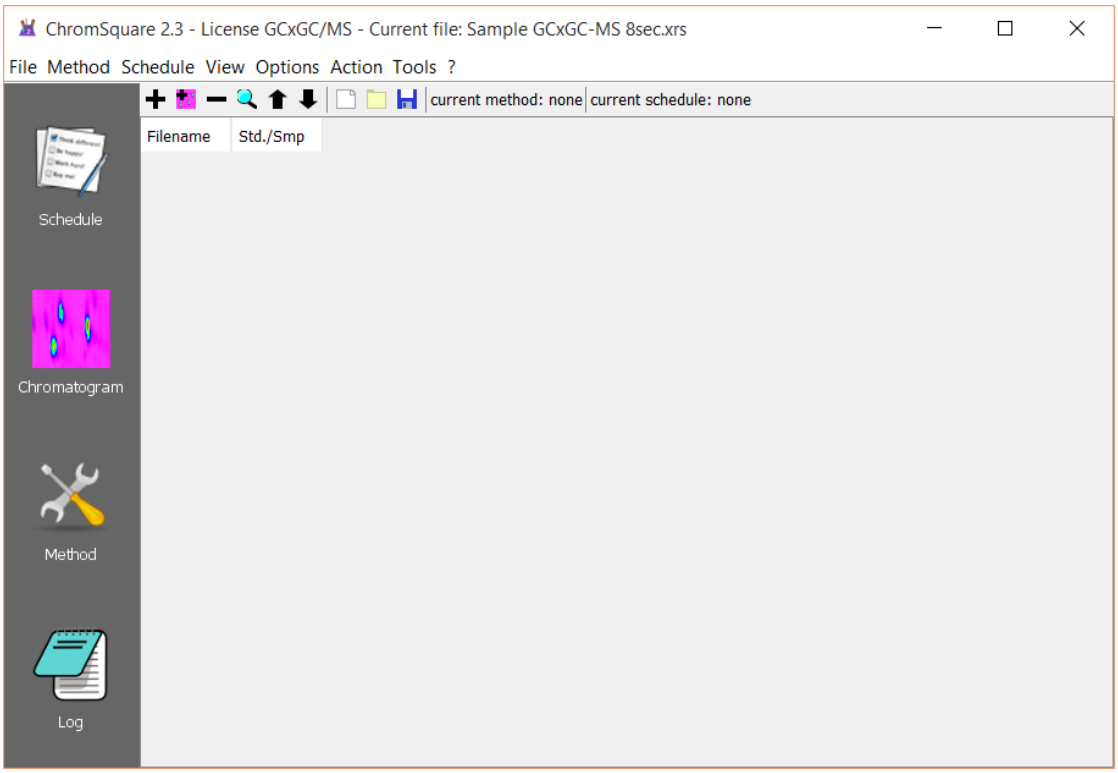


Figure 3.1: $Chrom^{square}$ Main Window

of instruments and to the software license installed, but all have a common peculiarity: **a Raw Data File is always treated *as is*; it cannot be changed or modified in any way**. Raw Data Files can only be read, copied or moved to a different folder.

- *Chrom*^{square} software uses for its purposes various files (schedule files, result files, Method files), which will be described later; we will call them generically *Chrom*^{square} *files*. All these files have a common peculiarity: they are all XML files, even if they do not have .xml extension, but a specific extension for each kind of file. Normally the user does not interact directly with these files: it is the software that manages them. However, being XML files means that they are open text files: if necessary, the user can explore their content using a normal browser, like Internet Explorer (or Mozilla or Safari or Google Chrome). A special mechanism (based on check-sum control) prevents the user to modify the file contents: *the user may inspect the files, but only the software may manage them.*

The *Chrom*^{square} files are described in the following paragraphs.

3.3.2 *Chrom*^{square} Result Files

Chrom^{square} Results Files are XML files which have "`.xrs`" extension (*Xml Result Set*).

They contain all possible information about the chromatogram, except chromatographic data, that remain stored in the Raw Data File (the reason of this choice is evident: chromatographic data can be really huge; there is no real benefit in the duplication of this amount of data for each chromatogram being analyzed; what is actually important is to maintain in the Result File a *link to the Raw Data file*).

Results File is a sort of summary of the relevant chromatographic data, useful for qualitative and quantitative analysis. It is a small file, that can be quickly read, avoiding the CPU overload due to reading of large raw data file when this is not necessary.

A Result File is generated as soon as a chromatographic file has been loaded with the Open Data File command (see 4.1.1); initially it contains only the link to the Raw Data File and few input information (Modulation Period, First Modulation Time) that have been set by the user at the opening of the file.

Subsequently, the Result File will be enriched with other information which reflect the progress of the analysis:

- after the integration completion, the Result File will include the list of all Blobs, with full details of each Blob;
- after the integration completion, the Result File will also include the full description of the Method used for the integration;
- if quantitative analysis is performed, the Results File will also include all results of the quantitation, namely the concentrations of the identified Blobs;
- the Result File also includes visual information (like axis scales and color scale), so that when the file will be reopened, it will reproduce the exact state of the analysis that there was at the instant of last saving.

The file is overwritten each time a relevant operation is performed on the chromatogram; file overwriting may be also forced by the user through the `Save Data File` command (see 4.1.1), in order to reflect minor changes.

3.3.3 *Chrom*^{square} Schedule Files

Chrom^{square} Schedule Files are XML files which have "`.wls`" extension (*Worklist Set*). A *schedule* (called also *worklist*), is a set of one or more chromatographic files.

The user defines a schedule by adding files to the table of the Schedule Panel (files may be also removed or moved up and down across the table).

Adding a file to the schedule is not the same thing that opening it: opening a file means loading into memory the full raw data, allowing the visualization of the chromatogram; *adding a file to the schedule just means to add an item to a list* (a special icon can be used to load and view each single chromatogram of the list).

Setting up a schedule give the user the possibility to plan operations to be accomplished on the whole set of chromatograms with just one command. These operations are:

- **Integration of a group of chromatograms** using the *same Method*; this operation is normally finalized to a calibration, but not necessarily; this operation is activated by the command `Action|Integrate All Chromatograms`, see 4.6.5.
- **Calibration**. Calibration is performed using the chromatograms of the schedule defined as *Standards*. A chromatogram is treated as a Standard if concentrations have been assigned for all Blobs defined in the Method.

3.3.4 *Chrom*^{square} Method Files

Chrom^{square} Method Files are XML files which have "`.xcm`" extension (*XML Chrom*^{square} *Method*).

Method Files are used for storing Method data (see 3.1.8).

3.3.5 Opening Files

When the user executes a command for opening a file (using the menu File Open command or the View icon of the Schedule Tool bar), the software executes some controls before actually opening the specified files:

1. if the user selects a Raw Data file, and *there is no corresponding Result* "`.xrs`" file in the same folder, the software opens it and generates the corresponding Result file;
2. if the user selects a Raw Data file, and *there is a corresponding Result* "`.xrs`" file in the same folder, the software displays a Dialog Box, which informs the user that opening the Raw Data will destroy the existing Result file;
3. if the user selects a Result "`.xrs`" file, the software loads from this file all available information, then searches the Raw Data specified in the Result file and loads it into memory;
4. the software cannot open a Raw Data file if there is in the same folder another Raw Data file with the same file name but different extension; in this case a warning message is displayed, and the user should move one of the two Raw Data files to another folder before continuing (this happens, for example, when an exported version - AIA/ANDI or any other version - of the same datafile had been generated using the same name).

3.4 The Java Console

This is a special window that does not belong to the main Graphical User Interface but that is shown in a separate window on the desktop. This windows is used by the software to record various kinds of informative messages. See Figure 3.2 for a sample of this window.

These messages contain technical information and are not designed for normal user, but for technical assistance.

The contents of the Java console greatly changes, according to the operations made by the user. The window automatically scrolls, like in traditional "Log" windows, always showing the most recent message. It is however possible to look at the oldest messages using the right scroll bar. It is also possible to clear the console in the case of too many messages.

Normally the Java Console is not shown. It should be used only in cases of malfunctioning of the software. There are two ways to show the Java console:

- Starting the software using the `Debug Mode` icon.
- Showing the window at run-time using the proper menu item (see 4.8.4).

Note that Java Console always is not automatically displayed as a full size window, but always starts in an iconized state. The user should explicitly maximize it to the normal size.

3.4.1 The Java Console buttons

On the bottom of the Java Console there are three buttons:

- **Copy to clipboard**. The contents of the window is copied to the clipboard (the clipboard contents can then be pasted on some other document).
- **Mail To**. The contents of the window is directly mailed to the technical service.
- **Clear**. The contents of the window is cancelled.

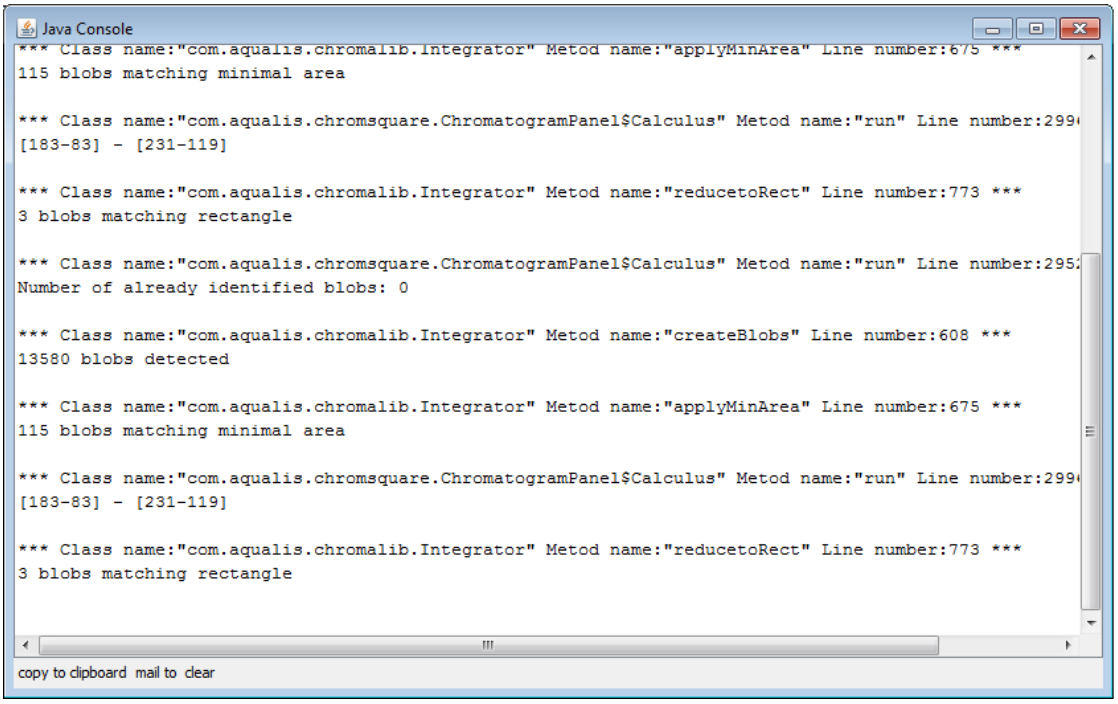


Figure 3.2: The Java Console

Chapter 4

Menu Bar

4.1 Menu `File`

The `Menu File` contains the main operations concerning chromatographic data files (opening, closing, saving). The menu is shown in 4.1

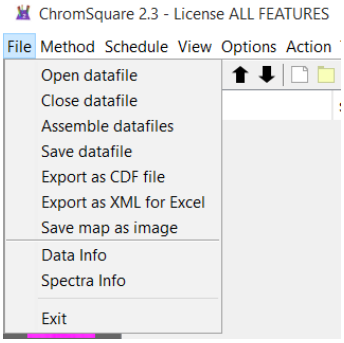


Figure 4.1: The Menu File

4.1.1 Open Datafile

This command is used for choosing a chromatogram and loading it directly into memory. The same operation can be performed through the icons of the Schedule Panel: in that case, however, the user first choose one or more chromatograms, and then load them into memory. The command Open Datafile, on the contrary, makes the two steps in a single operation (this command is useful when the user

wants to quickly check the contents of some chromatogram; the Schedule Panel procedure is recommended in the routine work).

The operation includes two steps:

1. **File selection**. A special File Selection Dialog Box is shown, to let the user to choose the file to be loaded.
2. **File loading**. The selected file is loaded into memory and shown in the chromatogram Panel; if everything goes well, a new row is added to the Schedule list. Otherwise, an error message is displayed and the Schedule list remains unchanged.

Read carefully the notes at Section 3.3.5 to fully understand the file mechanism.

4.1.1.1 File Types

The File Selection Dialog Box filters the directory contents and only shows the files which are compatible with the purchased license type.

The present manual applies to license GCxGC/MS & LCxGC/MS version for Shimadzu GCMSsolution and GCsolution.

This license is compatible with the following Data files:

- Shimadzu file types "`.gcd`" and "`.qgd`"; Shimadzu exported file types "`.aia`", "`.andi`", "`.cdf`", "`.csv`", "`.txt`"
- *Chrom*^{square} Result Data Files (file type "`.xrs`").

4.1.1.2 The Accessory Panel

The command `Open Datafile` shows an Open File dialog box, which is slightly different from the traditional ones; in fact it contains, on the right side, an `Accessory panel` (see the sample in the Figure 4.2).

This panel is made up by three parts:

- the *upper part* is used to define and select a *profile* (see Section 11.6); since profiles are defined only for some kinds of files, this part is not always displayed;

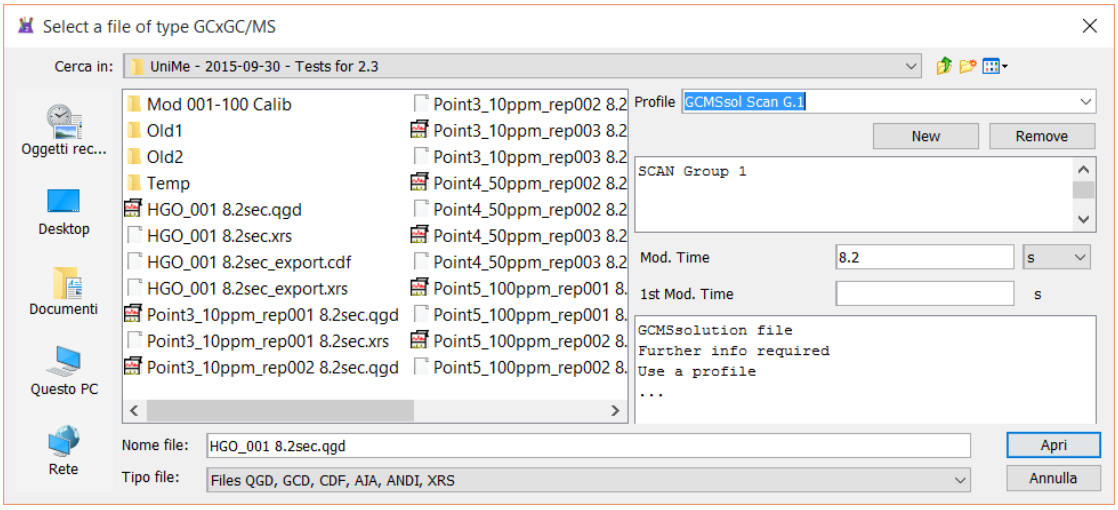


Figure 4.2: File description and features of the Data file selected

- the *central part* (that becomes the upper part when profiles are not used) is reserved to the *user*, which can specify some additional information about the data file being opened (modulation time, 1^{st} modulation time);
- the *lower* part is used by the *software* to display information about the data file (the software actually reads a little fragment of the file, trying to detect all information about its structure, and reports the obtained information in this part of the window; when this is not possible, information should be supplied by the user through a profile; in this case, the software warns the user with the message "`further information required: use a profile`").

4.1.1.3 Setting the Modulation Time

The Modulation Time must always be defined (otherwise the software does not know how to extract the modulations from the chromatogram).

Modulation Time can be assigned in two ways:

- *Explicitly*, by directly writing the value in the text field; note that the value entered in the field will be interpreted by the software according to the units (minutes or seconds) shown in the combo box on the right; if the units are changed after that the value has been entered, the value will be automatically converted to the new units; for this reason, the user should always choose the correct units *before* entering the Modulation Time value.

- Implicitly, by assigning to the data file a filename which contains the modulation time, according to the following rules:
  - The last part of the filename (before the file extension) should consist by a *number* followed by a *unit*;
  - unit can be "min" for minutes, or "sec" for seconds.

  For example: `2min` or `120sec`.

4.1.2 Close Datafile

This command closes the current data file, clearing all references to the chromatogram currently loaded.

4.1.3 Assemble Datafile

This command assembles more chromatograms into a unique data file; it is used when the modulations had been saved onto different files (a single file for each modulation, with files numbered as a sequence). See Section 11.9 for details.

4.1.4 Save Datafile

This command allows to save various information about the chromatogram being analyzed into a special *Chrom*^{square} file, called *Result File* (extension `.xrs`). See Section 3.3.2 for details about this file.

4.1.5 Export as CDF file

This command allows to export the current chromatogram in *NetCDF*[1] format, according to the *AIA-ANDI*[2] standards. The exported file will have "`.cdf`" ex-

[1] NetCDF (Network Common Data Form) is a set of software libraries and self-describing, machine-independent data formats that support the creation, access, and sharing of array-oriented scientific data.

[2] AIA-ANDI is a set of standards developed in 1990 by manufacturers of analytical instrumentation, through their trade organization, the Analytical Instrument Association (AIA). AIA sponsored the development of a series of standards to interchange analytical data across vendor platforms. These standards, known as ANalytical Data Interchange (ANDI) standards, are supported by many commercial software products. NetCDF files are *binary* files, that must read and written by means of specific software libraries; they cannot be managed by plain text editors.

tension (a suffix "_export" is also added to the file name before the extension, in order to avoid the presence of two files with same name and different extensions in the same folder, which is not allowed by the software).

4.1.5.1 Exporting a subset of the chromatogram

Starting from Release 2.3it is possible to export only a part of the chromatogram: this feature is particularly useful in the case of big files which require high loading times; the user can extract as a NetCDF file a smaller portion of the chromatogram containing the interesting blobs, and then continue the analysis on this reduced chromatogram.

This feature can also be applied to a set of chromatograms: see the command `Schedule|Export All Files As CDF`.

The portion of the chromatogram to be exported must correspond to an integer set of contiguous *modulations*; the selected modulations will be *entirely* exported, that is all data points along the vertical axis of the Map View will be exported.

In order to allow the user to specify a selection of modulations, an Input Dialog Box is shown, asking for the starting and ending modulations:

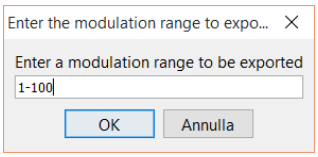


Figure 4.3: The Modulation Selection for Export CDF

The user must:

- Enter the two modulation values separated by comma (or minus sign or whitespace), and press the Ok button;
- Directly press the Cancel button, in order to bypass the modulation selection and export the whole chromatogram.

4.1.6 Export as XML for Excel

This command allows to save various information about the chromatogram being analyzed into a special "`.xml`" file, similar to the "`.xrs`" file (see 3.3.2).

The exported file has "`.xml`" extension; these files can be easily read and managed by the latest versions of Microsoft Excel, which allows a deep investigation of the file contents. XML files can also be viewed (with less functionality) by Internet Explorer or other Internet browsers.

The software always asks for the filename of the exported file and the folder where it should be placed (the folder path is remembered for future savings). It is a good practice to use filename and folders different from those of the original chromatogram, to avoid conflicts.

4.1.7 Save Map as Image

Any image displayed by the *Chrom*^{square} software can be saved as a graphic "`.png`" file.

The command opens a File Selection Dialog Box for the selection of the folder and of the file name of the graphic file.

The Dialog Box (represented in Figure 4.4) also contains some customization controls:

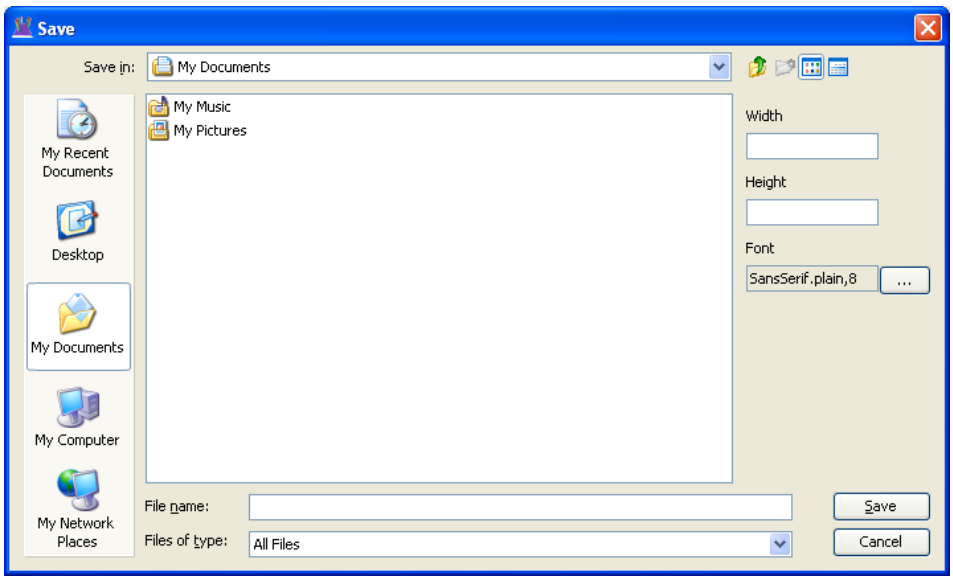


Figure 4.4: The Save Map as Image Dialog Box

- The text fields Width and Height, that can be used to specify the size of the image in *dpi*;

- the button with the three dots, which in turn opens a second dialog box for choosing the appropriate font and size (Figure 4.5).

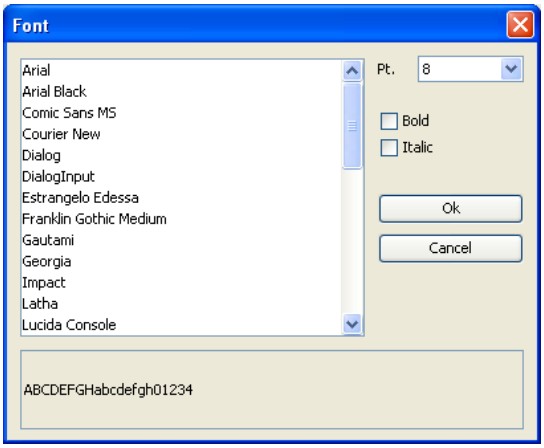


Figure 4.5: The Select Font Dialog Box

4.1.8 Data info

This command opens a Dialog Box which shows some information about the current chromatogram. A sample of the Data Info Dialog Box is shown in Figure 4.6.

This information is useful for checking purposes, but is not generally used during the normal routine analysis.

4.1.9 Spectra info

This command opens a Dialog Box which shows information about all spectra loaded in the current data file. The information includes Event Type, Group Number and relative number of data points. This information is particularly useful when the new feature of loading the whole set of spectra has been selected.

A sample of the Spectra Info Dialog Box is shown in Figure 4.7.

4.1.10 Exit

This command closes the current session of $Chrom^{square}$; a confirmation request is displayed, in order to avoid an accidental closure.

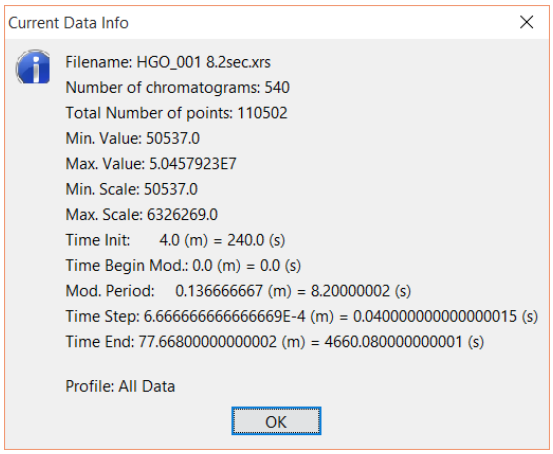


Figure 4.6: The Current Data Info Dialog Box

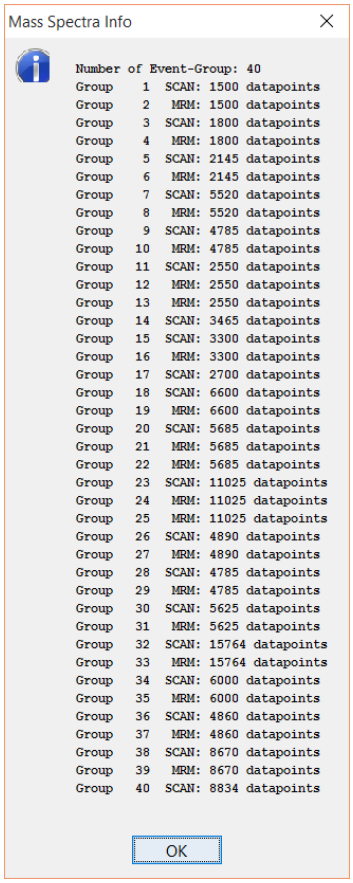


Figure 4.7: The Spectra Info Dialog Box

4.2 Menu `Method`

The `Menu Method` contains the commands for the management of the Method files (“`.xcm`”, see 3.3.4).

The menu is shown in Figure 4.8.

The commands `Open`, `Save`, `Save As` act like all traditional file management commands; the only peculiarity is that file names are filtered through the extension “`.xcm`”, and that the software keeps a record of the last folder used for storing Method files (so that it normally shows this folder when prompts the user for a Method file selection).

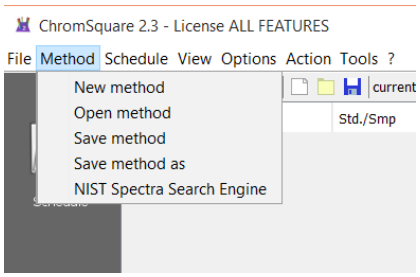


Figure 4.8: The Menu Method

4.2.1 New Method

The command clears the current Method and switches the current visualization (see 3.2) to the `Method Panel`. The Panel will show an empty Method (that is a Method with only some default parameters defined) that the user could fill, as described in Chapter 7.

4.2.2 Open Method

The command opens a File Selection Dialog Box, which prompts the user for the selection of an existing Method file (“`.xcm`” extension). As soon as the file is loaded, the visualization shifts to the `Method Panel`, that will show all the parameters of the Method just loaded.

4.2.3 Save Method

The command saves the Method that is being currently edited in the Method Panel, writing it onto a Method file.

If this Method had been previously loaded from a Method file through an `Open Method` command, the current Method will be saved onto the same file and folder. If no Method had been loaded, the software asks the user to use the command `Save Method As`.

4.2.4 Save Method As...

The command saves the Method that is being currently edited in the Method Panel, writing it onto a Method file.

In this case, the software always asks the user to select the name and the folder to be used for the Method file. The command is typically used when no Method file had been yet defined, but it can also be used to save an existing Method file with a different name.

4.3 Menu `Schedule`

The `Menu Schedule` contains the main operations concerning the management of the *Schedules*. See Chapter Section 5 for the description of the Schedule Panel, and Section 3.3.3 for a description of Schedule Files.

The menu is shown in 4.9

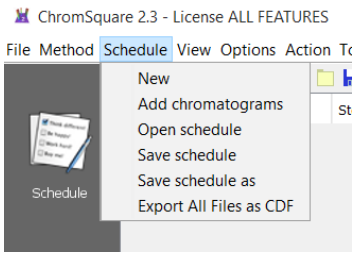


Figure 4.9: The Menu Schedule

4.3.1 New

The command will set up a new Schedule (that is an empty one, containing no chromatograms), that will become the *Current Schedule*.

4.3.2 Add chromatograms

The command opens a File Selection Dialog Box which allows the user to select one or more chromatograms to be added to the current Schedule. Pressing the CTRL key during the selection will allow to select more than one chromatogram.

The command performs the same action of the `Plus Sign` icon of the Schedule Toolbar (see Section 5.1). As already pointed out in that Section, the command does not actually load the chromatogram/s, but just creates the link/s for the subsequent analysis.

4.3.3 Open Schedule

The command opens a File Selection Dialog Box for the selection of an existing Schedule File ("`.wls`", see 3.3.3), that becomes the *Current Schedule*.

The file contents is copied in the Schedule Table (see 5.2), that can then be edited by the user.

The command does not automatically switch the current visualization to the Schedule Panel.

4.3.4 Save Schedule

The command saves the Current Schedule onto the Schedule File ("`.wls`") from which had been previously loaded.

If the Current Schedule is a New Schedule that had not been loaded from an existing file, a warning message is shown, asking the user to use the command Save Schedule As.

4.3.5 Save Schedule As...

The command opens a File Selection Dialog Box for the selection of a folder and of a file where the Current Schedule will be saved as a Schedule File ("`.wls`").

The command is used both for defining a new Schedule File and for overwriting an existing one.

4.3.6 Export All Files as CDF

This command is a generalization of the command `File|Export as CDF File`. It is used to convert the full set of files included in a Schedule as a single command.

Like in the case of the export of a single file, before executing the conversion the software asks for a range of modulations to be exported. This allows to reduce the sizes and the execution times of calibration sets made up of large data files.

See 4.1.5 for more details.

4.4 Menu `View`

The `Menu View` contains some commands concerning the visualization of the chromatogram.

The menu is shown in 4.10

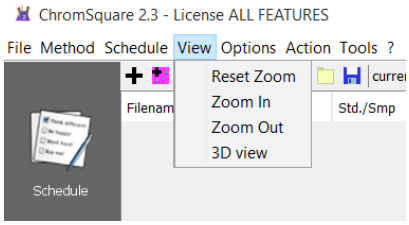


Figure 4.10: The Menu View

4.4.1 Reset Zoom

The command restores the initial visualization of the full chromatogram after one or more zoom operations.

See Section 6.2.3 for a complete description of Zoom operations.

4.4.2 Zoom In

This command increases the zoom factor by a fixed amount; the effect is to see a *smaller region* and *more details* around the current position of the mouse cursor.

See Section 6.2.3 for a complete description of Zoom operations.

4.4.3 Zoom Out

This command decreases the zoom factor by a fixed amount; the effect is to see a *larger region* and *less details* around the current position of the mouse cursor.

See Section 6.2.3 for a complete description of Zoom operations.

4.4.4 3D View

The command generates a 3D View of the region currently selected in the map plot.

See Section 11.1 for details about the 3D representation.

4.5 Menu `Options`

The `Menu Options` contains the commands which allow a fine tuning of the bi-dimensional plot (both the plot details and the drawing of various labels).

All these commands work as switches: each time that an item is selected, the switch changes from *ON* to *OFF* and back; when the switch is *ON*, a small *check* sign is shown to the left of the menu item.

The menu is shown in Figure 4.11.

The menu Options is divided in three parts, separated by horizontal lines:

- The first division contains switches regarding *interpolation*;
- The second part contains switches which set the details of the *2D map plot* (upper frame of the chromatogram panel);
- The third part contains switches which set the details of the *linear map plot* (lower frame of the chromatogram panel)

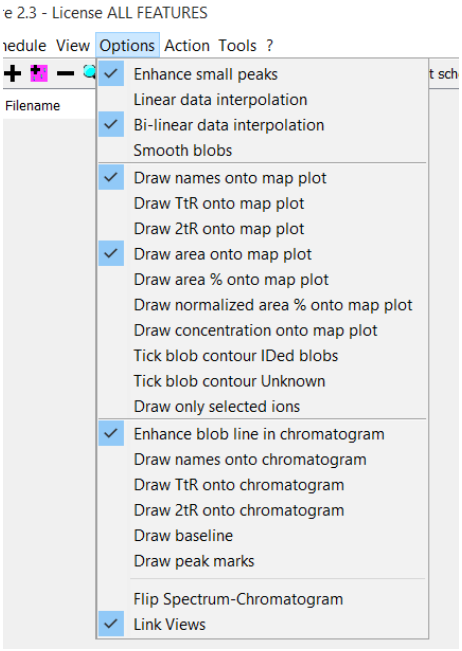


Figure 4.11: The Menu Options

4.5.1 Interpolation switches

4.5.1.1 Enhance Small Peaks

The switch enables an algorithm which enhances the visualization of the small peaks.

4.5.1.2 Linear Data Interpolation

The switch enables the *linear interpolation* of data on the 2D map: if **ON**, the absorbance value at any geometrical point of the map is calculated as the result of a linear interpolation of the values of the nearest chromatographic data points *along the vertical direction*, generating an effect of a continuous shade of colors only in that direction: in the other direction only the values of the actual chromatographic data points are taken into account, generating an effect of a "pixelated" map. Linear and Bi-linear interpolations are mutually exclusive.

4.5.1.3 Bi-Linear Data Interpolation

The switch enables the *bi-linear interpolation* of data on the 2D map: if **ON**, the absorbance value at any geometrical point of the map is calculated as the result of a bi-linear interpolation of the values of the nearest chromatographic data points in *both directions*, generating an effect of a *continuous shade of colors*.

4.5.1.4 Smooth Blobs

The switch enables the *smoothing* of the Blob contours: if **ON** the contours are bounded by *smoothed* curves, if **OFF** by *straight lines*.

4.5.2 2D Chromatogram Plot switches

4.5.2.1 Draw Names onto Map Plot

The switch enables the writing of the *names* of the Blobs in the bi-dimensional map plot.

4.5.2.2 Draw TtR onto Map Plot

The switch enables the writing of the *Total retention time* (First Dimension retention time + Second Dimension retention time) of the Blobs in the bi-dimensional map plot.

4.5.2.3 Draw 2tR onto Map Plot

The switch enables the writing of *Second Dimension retention time* of the Blobs in the bi-dimensional map plot.

4.5.2.4 Draw Area onto Map Plot

The switch enables the writing of the *Areas* of the Blobs in the bi-dimensional map plot.

4.5.2.5 Draw Area Perc onto Map Plot

The switch enables the writing of the *Area Percents* of the Blobs in the bi-dimensional map plot. The *Area Percent* of a Blob is computed as the percent ratio of the area of the Blob with respect to the sum of the areas of *all* Blobs (both identified and unidentified).

4.5.2.6 Draw Normalized Area Perc onto Map Plot

The switch enables the writing of the *Normalized Area Percents* of the Blobs in the bi-dimensional map plot. The *Normalized Area Percent* of a Blob is computed as the percent ratio of the area of the Blob with respect to the sum of the areas of all *identified* Blobs.

4.5.2.7 Draw Concentration onto Map Plot

The switch enables the writing of the *Concentrations* of the Blobs in the bi-dimensional map plot.

4.5.2.8 Tick Blob Contour IDed Blobs

The switch enables the drawing of the Blob contour of the *identified* Blobs in the bi-dimensional map plot.

4.5.2.9 Tick Blob Contour Unknown

The switch enables the drawing of the Blob contour of the *unidentified* Blobs in the bi-dimensional map plot.

4.5.2.10 Draw Only selected Ions

The switch enables the drawing of a bi-dimensional map plot based upon a *selection of ions* instead of the normal map plot based on *all ions*. The selection of ions is defined in the Method (see Section 7.2.7.2).

4.5.3 Linear Chromatogram Plot switches

4.5.3.1 Enhance Blob Line in Chromatogram

The switch enables an enhanced representation of the Blob line contour for a better visualization in the linear chromatogram view.

4.5.3.2 Draw Names onto Chromatogram

The switch enables the writing of the name of the Blob which the peak belongs to in the linear chromatogram view.

4.5.3.3 Draw TtR onto Chromatogram

The switch enables the writing of the total retention time of the top of the Blob which the peak belongs to in the linear chromatogram view.

4.5.3.4 Draw 2tR onto Chromatogram

The switch enables the writing of the second dimension retention time of the top of the Blob which the peak belongs to in the linear chromatogram view.

4.5.3.5 Draw baseline

Enables or disables the drawing of the *baseline* of the peaks in Chromatogram view.

4.5.3.6 Draw peak marks

Enables or disables the drawing of the *start/end marks* of the peaks in Chromatogram view.

4.5.3.7 Flip Spectrum-Chromatogram

Sets the upper right view to *Chromatogram View* and the lower left view to *Spectrum View* and vice-versa.

4.5.3.8 Link Views

Enables or disables the synchronization of Map View and other views while moving the mouse in the Map View. When synchronization is disabled, the user may move the mouse in the Map View without any change in the other views. The side views are updated only when the user left clicks the mouse at the desired position.

4.6 Menu `Action`

The commands of the menu Action correspond to the actions that the user can perform on the software, like various kinds of integration, Blob searching, calibration, quantitation and so on.

The menu is shown in 4.12

Note: Figure 4.12 shows a full menu Action; some menu items are not implemented in all software versions. The items implemented in your license version are those described in the following of the current section.

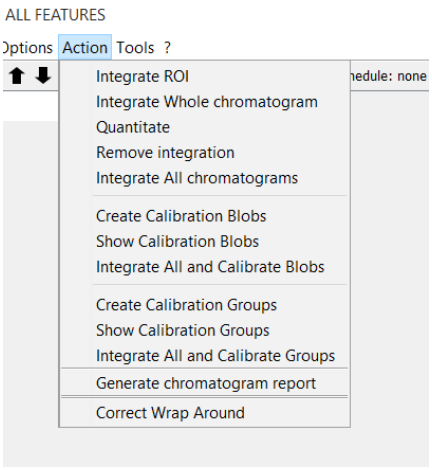


Figure 4.12: The Menu Action

4.6.1 Integrate ROI

The command performs the integration on a *Region of Interest* (ROI), that is on only a part of the chromatogram plot (see Section 3.1.3 for the exact meaning of *integration*). ROIs are useful to speed up the calculation, especially in the case of large chromatograms.

The ROI is stored within the Method (see Section 7.2.7.1) and can be graphically defined through the mouse and context menu command (see Section 6.2.4.3).

See the next Section (4.6.2) for details about the integration process and results.

4.6.2 Integrate Whole Chromatogram

The command performs the integration of the whole chromatogram plot (see Section 3.1.3 for the exact meaning of *integration*).

The integration can take some time, during which a progress bar, showing the advancement of the calculation, may be displayed. At the end of the integration the following changes will appear to the user:

- In the Map Plot window, all recognized Blobs are marked and tagged with various kinds of information, according to the options set up through the Menu Options (see 4.11).
- The Table of the Results page (in the lower window of the Chromatogram Panel) will be filled with the integration results.

4.6.3 Quantitate

The command starts the execution of the *Quantitative Analysis* (also called *Quantitation*). See Chapter 9 for a detailed description of the procedure.

Since Quantitation requires that a *Calibration* has already been set and saved on a Schedule file (see Section 3.3.3), the first action subsequent to the Quantitate command is a File Selection Dialog Box that will ask for the Calibration Schedule file.

4.6.4 Remove Integration

The commands clears the current integration.

The Map Plot will be cleared by Blob marks and labels, and the Table of the Result page will be emptied.

This command is seldom used, since each integration (both *Whole* and *ROI*) overwrites the former one.

4.6.5 Integrate all chromatograms

The command starts a procedure for the automatic integration all the chromatograms in the schedule list.

The process, once initiated, cannot be stopped by the user. In the case of huge chromatograms, the integration of all chromatograms may take a long computation time: it is then a good practice, when dealing with a new schedule, to execute a single integration (see 4.6.2 to get an estimate of the overall computation time.

4.6.6 Create Calibration Blobs

The command starts the procedure for the generation of a new *Calibration* curve, based on the current schedule and using Blob areas for calculation.

See Section 9.1.10 for a detailed description of the procedure.

4.6.7 Show Calibration Blob

The command opens a new window where are summarized the features of the current *Calibration based on Blobs* (if it has already been defined).

4.6.8 Integrate All and Calibrate Blobs

This command executes sequentially the operations of the commands `Action|Integrate All Chromatograms` and `Action|Create Calibration Blobs`.

The command is useful in routine task, since it allows to start a lengthy operation with a single action.

4.6.9 Create Calibration Group

The command starts the procedure for the generation of a new *Group Calibration* curve based on the current schedule and using Group areas for calculation.

See Section 9.4.4 for a detailed description of the procedure.

This command is visible only if the current Method contains one or more Group.

4.6.10 Show Calibration Group

The command opens a new window where are summarized the features of the current *Calibration based on Groups* (if it has already been defined).

This command is visible only if the current Method contains one or more Group.

4.6.11 Integrate All and Calibrate Groups

This command executes sequentially the operations of the commands `Action|Integrate All Chromatograms` and `Action|Create Calibration Groups`.

The command is useful in routine task, since it allows to start a lengthy operation with a single action.

This command is visible only if the current Method contains one or more Group.

4.6.12 Generate Chromatogram Report

The command generates a report on a PDF file.

Reports can containing both the chromatogram map and the table of results, with different levels of details which are assigned through a *Report Template*[3].

Some standard Report Templates are supplied at installation time. Other templates may be created by the user, using the tool Report Template Editor (see Section 11.5).

As first action, the Create Chromatogram Report command displays a pop up window, showing the list of available Report Templates (see Figure 4.13).

After that the user has chosen the desired template, the relative report is generated and displayed in a Preview window. A sample of this window is shown in Figure 4.14

[3]The Report Templates are XSL files, that is XML text files which follow the "XSL Transformations (XSLT) Version 2.0" rules, see `http://www.w3.org/TR/xslt20/`.

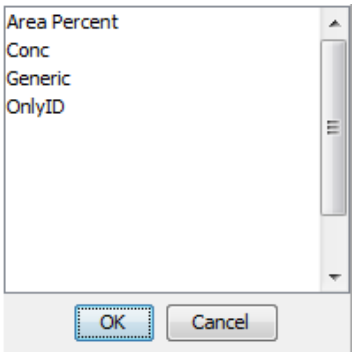
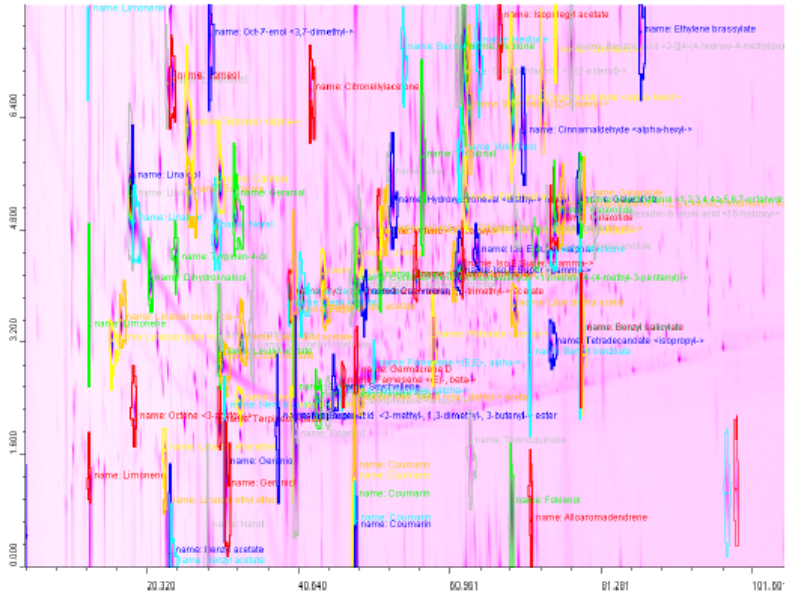


Figure 4.13: The Report Template Selector Dialog Box

Chromatogram Report



Blob Name	TtR	2tR	Area
unknown	4.008	0.008	11138683.4
Limonene	12.532	0.132	7424521.8
Limonene	12.554	0.021	12326288.6
Limonene	12.590	0.057	13236115.0
Linalool oxide <cis->	15.654	0.054	17064640.6
Linalool oxide <cis->	16.859	0.059	15110322.9
Linalool	18.488	0.088	1400676747.9
Linalool	18.492	0.092	241687437.5
Linalool	18.616	0.082	16719942.0
Octene <3-acetoxy->	18.702	0.035	14300792.2
Dihydrolinalool	20.735	0.068	42486605.1
Linalool ethyl ether	22.694	0.028	18147053.4
Linalool ethyl ether	22.815	0.015	7086734.8
Lavandulol	23.182	0.115	12481074.1
Benzyl acetate	23.470	0.003	102273925.5
Benzyl acetate	23.601	0.001	11372425.0
Borneol	23.849	0.116	27326860.5
Terpinen-4-ol	24.340	0.073	56724782.9
Terpineol <alpha->	25.838	0.105	94532457.7
Estragole	26.089	0.089	65342969.9
Nerol	28.409	0.009	9432852.0
Oct-7-enol <3,7-dimethyl->	28.792	0.126	26483227.7

Figure 4.14: The Chromatogram Report Preview

4.6.13 Search selected Blob

The command calls the instrumentation software in order to search the *selected Blob* (use the Data Analysis context menu, described at Section 6.5.4, to select the Blob).

This command is presently implemented only for GCMSsolution, LCsolution and LCMSsolution versions of the software.

4.6.14 Search all Blobs

The command calls the instrumentation software in order to search *all Blobs*.

This command is presently implemented only for GCMSsolution, LCsolution and LCMSsolution versions of the software.

4.6.15 Correct Wrap Around

This command allows to add a time interval to the *start modulation time*: in this way all modulations are shifted by the same time amount; the visual result is that the map will appear shifted towards the top or towards the bottom, with a *wrap* effect.

4.7 Menu `Tools`

The `Menu Tools` contains commands which calls some auxiliary modules, which perform some *independent* operations (that is operations not linked to the current loaded chromatograms).

The menu is shown in 4.15

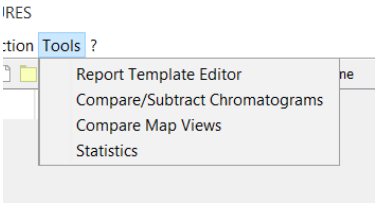


Figure 4.15: The Menu Tools

4.7.1 Report Template Editor

This command calls a special tool, the *Report Template Editor*. The tool is described in detail in Section 11.5

4.7.2 Compare/Subtract Chromatograms

The command opens a special window designed to perform the comparison and subtraction of two *one-dimensional* chromatograms.

The tool is described in detail in Section 11.2.

4.7.3 Compare Map Views

The command opens a special window designed to perform the comparison and subtraction of two *bi-dimensional* chromatograms.

The tool is described in detail in Section 11.3.

4.7.4 Statistics

The command opens a special window which allows to perform various kinds of statistical analysis on a set of integrated chromatograms.

The tool is described in detail in Section 10

4.8 Menu `Help`

The `Menu Help` is identified by a question mark and - as usual - is the last menu of the Menu Bar.

The menu contains items relative to various kinds of information to be displayed.

The menu is shown in 4.16

4.8.1 Help

The command shows the $Chrom^{square}$ User Manual in an Acrobat Reader window.

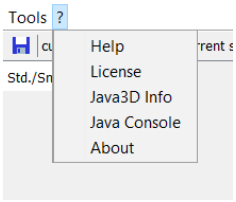


Figure 4.16: The Menu Help

4.8.2 License

The command displays information about the license currently installed.

4.8.3 Java 3D Info

This command opens a window that displays information about the Java 3D version installed (if any). This information is useful when the 3D visualization command (see 11.1) does not generate an image or the image is not satisfactory: in these cases, the information displayed should be sent to the Technical Support in order to detect the origin of the problem. A sample window is shown in Figure 4.17.

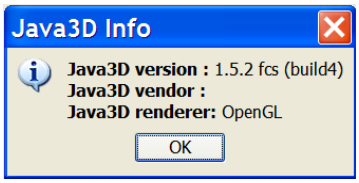


Figure 4.17: The The Java 3D information window

4.8.4 Java Console

This command opens a new window, called *Java Console*. Java Console window is used by the software to record various information that can be useful to the technical assistance in the cases of malfunctioning of the software. See Section 3.4

Normally, this window is shown initially if the user choose to start $Chrom^{square}$ in *Debug mode*; the command lets the user to show the window at any moment at run time.

4.8.5 About

The command displays information about $Chrom^{square}$ current version and references about the software producer.

Chapter 5

Schedule Panel

The Schedule Panel is shown when the user clicks on the corresponding large icon `Schedule` of the Side Bar Panel (see Figure 3.1)

The Schedule Panel contains a *Schedule Bar* and the *Schedule Table*.

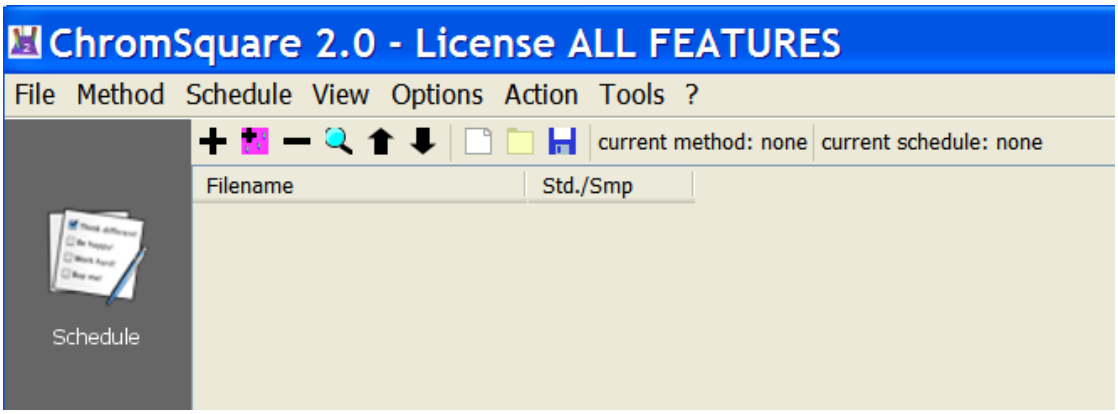


Figure 5.1: The Tool Bar of the Schedule Panel

5.1 Schedule Tool Bar

The Schedule Tool Bar is placed in the upper side of the *Chrom*^{square} main interface, immediately under the Menu Bar. See Figure 5.1.

This Tool Bar is shown only when the Schedule Panel is selected. The icons of the Tool Bar summarize the most frequent operations; some of these operations correspond to menu commands; other ones are exclusive of the Tool Bar. The

meaning of each icon is also shown as a *tooltip* (small informative yellow window, which is automatically displayed when the mouse passes over or near the icon).

Scanning the icons of the tool bar from the left to the right, you can find the following commands:

1.  `Plus Sign`: Adds a Chromatogram to the current Schedule; does not open the chromatogram; just add it to the list. A File Selection Dialog Box appears, allowing the user to select the chromatogram.

2.  `Small Plus Sign over a coloured background`: Adds the current opened Chromatogram (if any) to the current Schedule.

3. `Minus Sign`: Removes a Chromatogram from the current Schedule; the command does not actually delete any file; only file reference is deleted from the current list.

4.  `Magnifying Lens`: Loads the selected chromatogram from the Raw Data file.

5.  `Arrow Up`: Moves the selected chromatogram one row up.

6.  `Arrow Down`: Moves the selected chromatogram one row down.

7.  `"New" symbol`: Creates a new empty Schedule. Corresponds to the menu command 4.3.1.

8.  `"Open" symbol`: Opens an existing Schedule. Corresponds to the menu command 4.3.3.

9.  `Floppy Disk symbol`: Saves the current Schedule. Corresponds to the menu command 4.3.5.

10. Text fields `"Current Method"` and `"Current Schedule"`.

The last item in the tool bar shows the name of the current Method (if a Method has already been loaded or defined).

5.2 Schedule Table

The Schedule Table is a table which shows *a row for each chromatogram loaded.*

The Schedule Table is a dynamic table, that is its contents vary according to the operations performed on the software.

The Table has at least two columns:

- the *first* column contains the filenames of the chromatograms;
- the *second* column is used to define the role of the chromatogram in the calibration procedure (quantitative analysis, see 9): that is, if it is either a *standard of a given level* or an *unknown sample* (the column header reports the abbreviation `Std./Smp.`).

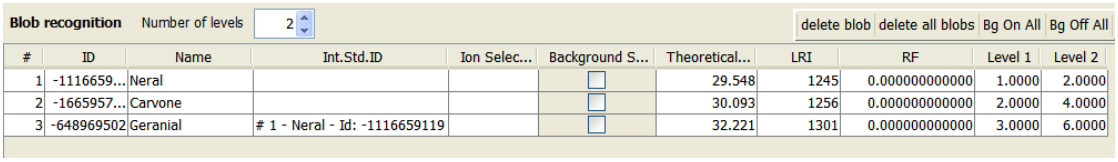
Subsequent columns correspond to the Blob IDs defined in the Method loaded.

5.2.0.1 Populating the Schedule Table

The population of the table evolves as follows:

1. Initially (when software starts or the command `New Schedule` is executed), the table only contains the header of the first two columns (“`Filename`”, “`Std./Smp.`”) and no rows, exactly as shown in Figure 5.1.
2. The first operation to be done on a New Schedule is *to define the Method associated to the Schedule*. This is normally accomplished by loading an existing Method with the menu command `Method|Open`. If the Method has not yet been defined, define it now (see section 7), save it assigning a Method name and then load it.

   In Figure 5.2 there is a snippet of the Method used to explain this section: the Table contains 3 Blob IDs and 2 Standard Levels; one of the Blob ID (Geranial) references another one (Neral) as Internal Standard.



#	ID	Name	Int.Std.ID	Ion Selec...	Background S...	Theoretical...	LRI	RF	Level 1	Level 2
1	-1116659...	Neral			☐	29.548	1245	0.000000000000	1.0000	2.0000
2	-1665957...	Carvone			☐	30.093	1256	0.000000000000	2.0000	4.0000
3	-648969502	Geranial	# 1 - Neral - Id: -1116659119		☐	32.221	1301	0.000000000000	3.0000	6.0000

Figure 5.2: A sample Method, with 3 Blobs, 2 Levels and 1 IS

3. As soon as the Method has been defined, the Schedule Table is filled with new columns, *each column corresponding to a Blob ID* defined in the Method. The new columns will have headers that display the names of the Blob IDs, as shown in 5.3.

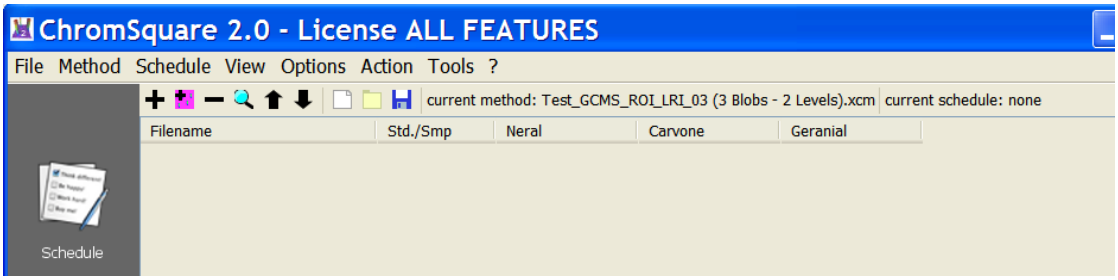


Figure 5.3: The Schedule Table with Headers

These columns are designed to store the *standard concentrations* of the compounds corresponding to the Blob IDs: a cell placed at a given row and column will contain the concentration of the compound corresponding to the Blob ID specified by the column, in the chromatogram specified by the row. This of course is only possible for the cells that correspond to external standards or internal standards; all other cells, corresponding to unknown samples, are initially set to 0.0, and will assume the calculated concentration values after the Calibration and Quantitative Analysis.

4. As long as chromatograms are added to the schedule list, new rows are added to the table, displaying the chromatogram filename in the first column of the table.

   In the present example, we add three chromatograms. See Figure 5.4.

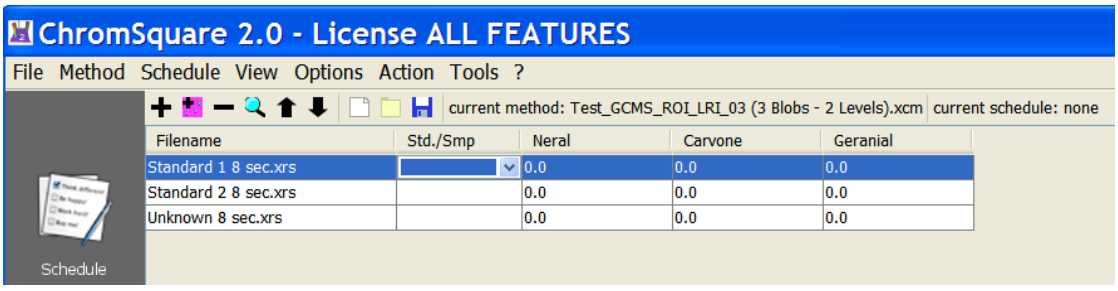


Figure 5.4: The Schedule Table with Headers and Chromatogram names

5. For each chromatogram inserted in the Table, the user should then choice the function of the chromatogram in the Calibration through the combo box

of the second column. The combo box will contain the following items (see Figure 5.5:

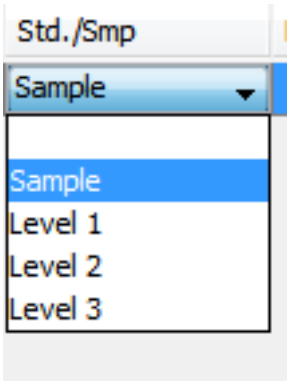


Figure 5.5: The Std/Smp combo box

- The *Sample* item;
- An item for each Standard Level defined in the Method, labelled as *Level 1*, *Level* 2, ...

In the current example, the first two chromatograms are defined as Standards (both of Level 1), while the third will be treated as Sample.

6. Soon after the choice, the software will fill and colour the cells in different ways, according to the following criteria:

   - If the user selects a *Standard Level*:
     - The cell of the combo column will display the Level and will be coloured in *Green* (meaning *Standard*).
     - All other cells of the row will display the concentrations of the Blob IDs, defined in the Method. These cells will be coloured in *Gray*, meaning that the user cannot modify the values.
   - If the user selects *Sample*:
     - The cell of the combo column will display the *Sample* label and will be coloured in *Yellow* (meaning *Sample*).
     - The cells of the other columns will behave differently depending on whether the Blob IDs is referenced as Internal Standard or not:
       * For the columns corresponding to Blob IDs referenced as Internal Standard, the cells will be coloured in *Green* (meaning

Standard) and is *editable*: the user should enter the concentration of the compound corresponding to the Blob ID used as Internal Standard in this chromatogram.

* For the columns **not** corresponding to Blob IDs referenced as Internal Standard, the cells will be coloured in Gray (meaning *not editable*), and the contents will remain *empty*.

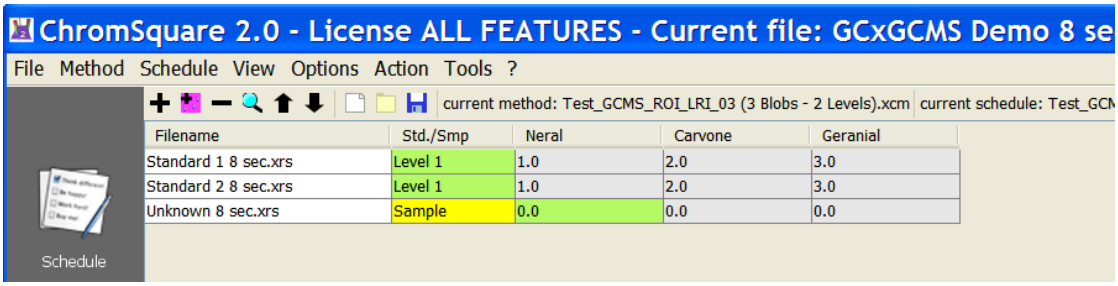


Figure 5.6: The Schedule Table after Standard Level/Sample assignment

5.2.0.2 Calibration

The Figure 5.6 shows the Schedule Table after the assignment. Note that in the Sample chromatogram the first Blob ID (Neral) is *green*, since it is referenced as Internal Standard. This means that the user should enter a suitable value in the cell (which is editable) before saving the Schedule. An error message will be displayed if a value is not entered.

Summarizing:

- For the combo column:
  - **Green** means *Standard* (*External*)
  - **Yellow** means *Sample*
- For the Blob ID columns:
  - **Grey** means *Not Editable*
  - **Green** means *Internal Standard (Editable)*

When one or more rows have been defined as standard (that is, all concentrations have been assigned) it is possible to proceed with the *Calibration*. If Calibration succeeds, the software automatically calculates the concentrations of the Blobs of

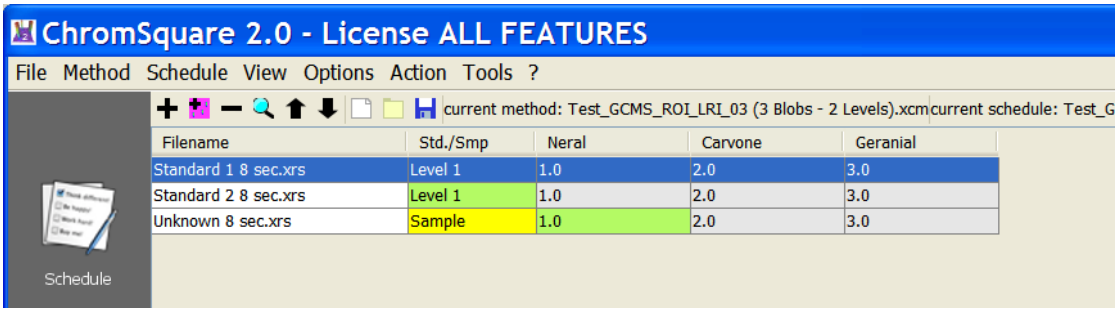


Figure 5.7: The Schedule Table after Calibration

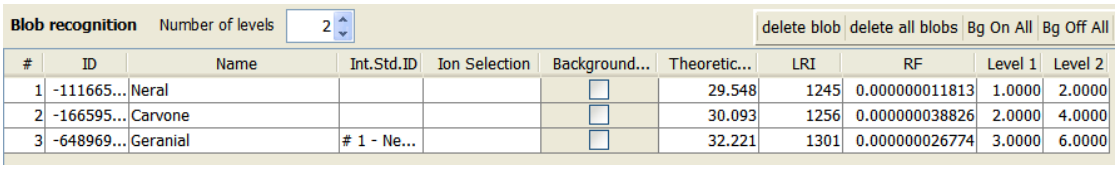


Figure 5.8: The Method Panel after Calibration

the sample chromatograms and fills the corresponding rows with the calculated values.

Figure 5.7 shows the Schedule Table of the current example after the Calibration. Note that also the row corresponding to the sample chromatogram now contains concentration values (assigned by the user, in *green*, or calculated, in *gray*).

The Calibration also updates the Method, inserting the values of the Response Factor: see Figure 5.8.

5.2.0.3 Saving and retrieving Schedules

A Schedule Table can be saved on a Schedule File (extension `.wls`), using the `Save` icon of the Schedule Tool Bar or the corresponding Menu command.

When a Schedule File is opened, the Schedule Table is automatically filled with all information saved on the file.

Chapter 6

Chromatogram Panel

6.1 Panel Contents

The Chromatogram Panel is made up of *four sub-panels or* ***Views*** and a *status bar*, as shown in Figure 6.1.

1. The *Map View*, in the *upper-left* (North-West) corner of the Panel. This window shows the Chromatogram as a coloured Map, also called *Contour Map*; it corresponds to the 2D representation of the chromatogram. It is described in Section 6.2.

2. The *Chromatogram View*, in the *lower-left* (South-West) corner of the Panel. It in turn is a multipl is contains various *sub-windows* or *pages* that can be selected using the upper *tabs*:

   - *Graphical Parameters* page (see Section 6.3).
   - *Chromatogram* page (see Section 6.4).
   - *Data Analysis* page (see Section 6.5).
   - *Grouping* page (see Section 6.6).

3. The *Spectra View*, in the *upper-right* (North-East) corner of the Panel. See Section 6.7.

4. The *Navigator View*, in the *lower-right* (South-East) corner of the Panel. See Section 6.8.

5. The *status bar* (see Section 6.9) displays information about the current position of the mouse cursor inside the upper Map View.

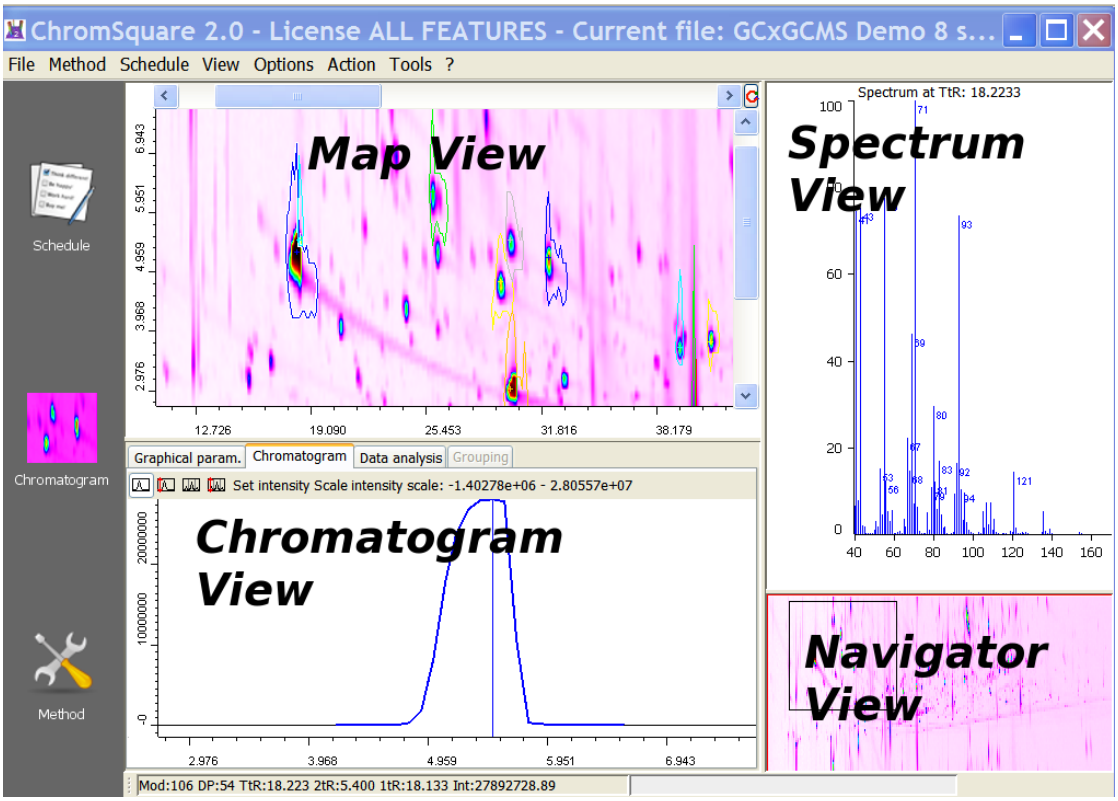


Figure 6.1: Chromatogram Panel Overview

The sizes of the four windows may be changed by the user through three *dividers*; these are moving bars that can be dragged by the user to resize the windows according to the needs:

- a *vertical divider* separates the left sub-panels by the right ones.
- a first *horizontal divider* separates the upper from the lower sub-panel in the *left* side.
- a second *horizontal divider* separates the upper from the lower sub-panel in the *right* side.

6.2 The Map View

6.2.1 Features

6.2.1.1 Graphics

The upper Map View contains a *Contour Map* which corresponds to the 2D representation of the chromatogram, namely:

- the *abscissa* axis represents the *Total Retention Time*; this is always expressed in *minutes*;
- the *ordinate* axis represents the *Modulation Time*; this is expressed in *minutes* when its value is greater then 2 minutes, otherwise it is expressed in *seconds*.

Let's remember that:

- a chromatographic point corresponds to a pair of numerical values, i.e. *time* and *intensity* (absorbance);
- in 2D chromatography the whole chromatogram is divided into a set of *modulations*, according to a *modulation time*; in this representation, the *time value* of the chromatographic point corresponds to two time values: the overall time value, represented along the *x-axis*, and the time value of the single modulation, represented along the *y-axis*;
- the *intensity value*, or absorbance, corresponds to the third dimension; in the 3D representation (see 11.1) this corresponds to the *z-axis*, whereas in the 2D representation this corresponds to a *color level*; the whole set of all points generates a *Contour Map*.

6.2.1.2 Operations

The Map View supports several kinds user interactions through mouse operations:

1. *Selecting* a region with the *left* mouse button: this directly causes a *Zoom* of the selected region.
2. *Selecting* a region with the *right* mouse button: this generates a *Map View Selection Context Menu*, which allows a further choice of operations. See section 6.2.4.

3. *Clicking on a point* with the *right* mouse button: this generates a *Map View Point Context Menu*, which allows a further choice of operations. See section 6.2.5.

Note the difference between the two types of context menu: both are obtained using the right button, but the first one ("Selection") is generated releasing the button after that a region has been selected dragging the mouse, whereas the second one ("Point") is generated releasing the button after a simple click.

6.2.2 Selection

When a new chromatogram is loaded into memory, the corresponding Contour Map of the *full chromatogram* is displayed in the Map View.

The user can then select through the mouse a smaller a smaller region of the Contour Map in a way that it well be represented in the whole Map View: this action is typically called *Zoom* and yields as a result an enlargement of the selected region, allowing to see more details. Zooming can be handled in different ways and is described in 6.2.3.

The selection af a smaller region of the chromatogram can be used not only for zooming but also for other operations concerning *regions*.

The selection operations are performed according to the common rules of most software applications:

- Click on a point of the plot with a mouse button to start selection (that is, the point becomes first corner of a moving rectangle);
- Move the mouse holding the button pressed to change the size of the rectangle; the mouse pointer identifies the opposite corner of the moving rectangle; the moving rectangle is shown in *reverse* colors;
- Release the mouse button to close the operation.

In $Chrom^{square}$ software the following rule will also apply:

- when the selection is made by dragging the mouse with the *left* button clicked, the result is directly the *Zoom In* of the selected region;
- when the selection is made by dragging the mouse with the *right* button clicked, the result is the displaying of a pop-up *context menu*, which allows

the choice among *different operations* to be made on the selected region (Zoom In is however provided even in this case); see Section 6.2.4 for a full description of this menu.

The Figure 6.2 shows a Map View selected region and the Map View Selection Menu.

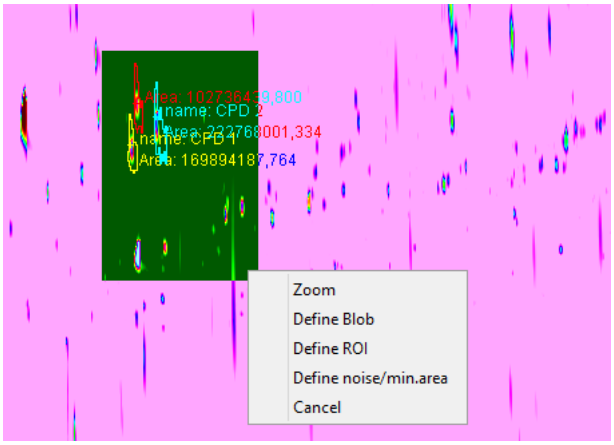


Figure 6.2: Map View - Selection Context Menu

6.2.3 Zoom

The Zoom In operations are generally performed by selecting a region through the mouse selection, as mentioned above. However, there are other Zoom operations (like Zoom Out, Reset Zoom or Move Zoom) that can not be easily accomplished in this way. For this reason, the software provides two other ways to perform Zoom operation, which complement the mouse ones:

- Zoom operations through the `Menu View` commands;
- Zoom operations through the *Zoom Mapping facility*, which is provided in a special window (*Navigator View*) of the lower panel (see 6.8 for a full description).

The Figure 6.3 shows the Map View after Zoom In operation, and the representation of the zoomed region in the Zoom Mapping facility. This is the same setting of 6.2 after that the first menu item (Zoom) has been selected.

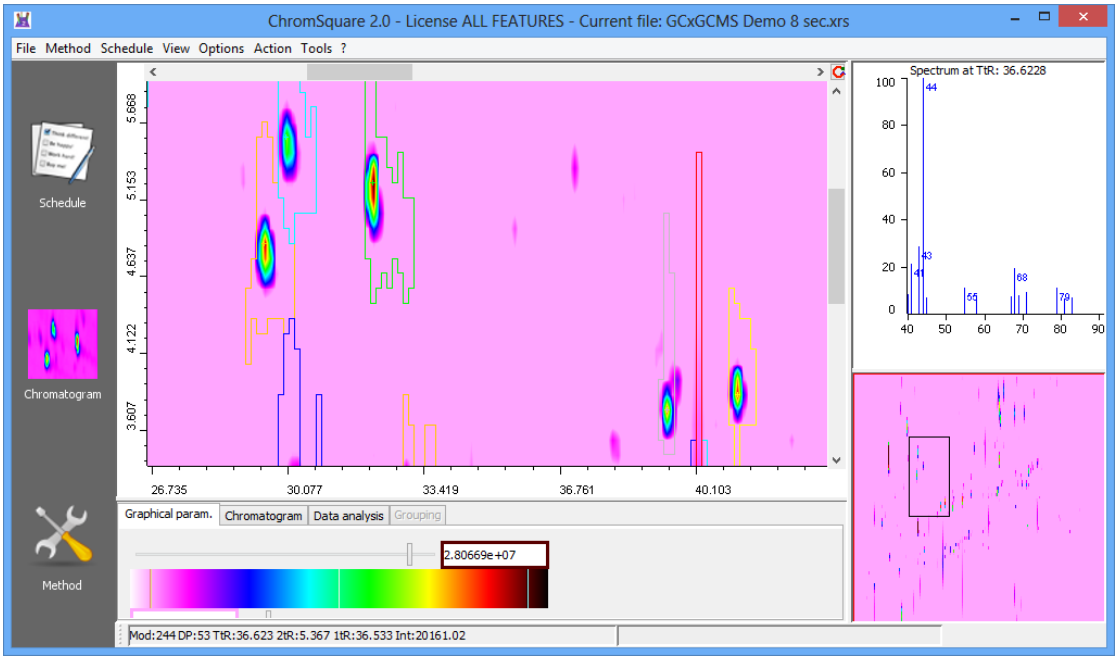


Figure 6.3: Map View - Zoomed Region

6.2.3.1 Zoom In

The *Zoom In* operation may be performed in *three* different ways:

- by selecting a region through mouse dragging with the *left button*: the region will be zoomed as soon as the button is released; this is the preferred way;
- by selecting a region through mouse dragging with the *right button* and then selecting `Zoom` from the menu context that will be shown as soon as the button is released; this way is slightly more complex, and it is provided only to facilitate the user;
- by using the menu command `View | Zoom In`; this is equivalent to select a zoom region having *half sizes and the same centre* with respect to the current plot.

The Zoom In operation may be performed more times, obtaining always more deep enlargements.

6.2.3.2 Zoom Out

The *Zoom Out* operation can be performed in *only one* way:

- by using the menu command `View | Zoom Out`; this is equivalent to select a zoom region having *double sizes and the same centre* with respect to the current plot.

6.2.3.3 Reset Zoom

The *Reset Zoom* operation can be performed in the following way:

- by using the menu command `View | Reset Zoom`; this causes the redrawing of the whole chromatogram, clearing all previous zoom operations.

6.2.4 Map View Selection Context Menu

This context menu is displayed after that a rectangular region of the Map View has been selected using the right mouse button. This rectangular region represents the *domain* to which the operation of the selected menu item will apply.

The context menu (shown in Figure 6.2) contains the menu items described in the following Sections.

6.2.4.1 Zoom

A *Zoom In* operation will be performed on the selected region. See 6.2.3.1 for a full description of Zoom In operation.

6.2.4.2 Define Blob

The menu item command is used to manually identify one or more *Blobs*, that is to assign a *Blob ID* to the Blob (or Blobs) contained in the selected region. If the region contains more Blobs, the same Blob ID will be assigned to all them.

As described in 3.1.6, a Blob ID is a data structure which contains a numerical *Id*, a *name* and a *geometrical description*. Whilst the Id is automatically generated by the software, and the description is given by the selected region, the name should be explicitly supplied by the user.

The assignment of the Blob name works as follows:

- Select with the right mouse button a region containing one or more Blobs, as described in 6.2.2.
- When the context menu appears, select the second item (Define Blob); a small window, with title `Insert Blob Name` will be displayed. The window contains a label which reports the initial and final *Total Retention times* of the selected region, and a text field for entering the name.
- Enter the desired Blob name in the text field and give `Ok` o confirm the assignment, or `Cancel` to delete the current assignment.

The window is shown in Figure 6.4.

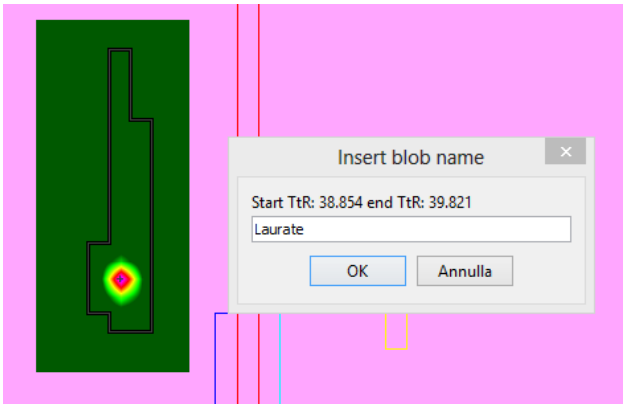


Figure 6.4: Insert Blob Name window

It is also possible to define a Blob ID whose region is a *polygon* instead of a simple rectangle (this feature is called *Polygonal Blob area*; see Section 11.4 for details). A rectangular region becomes in this way a particular case of a polygonal one.

The new Blob Id with all its data is automatically inserted in the current Method.

The user can at once verify the Blob name and Id by switching the view to the Method Panel[1].

Figure 6.5 shows a fragment of the Method Panel after that the new Blob ID has been defined.

[1]The geometry is also stored in the Method, but it cannot be seen in the Method Panel due to the complexity arising from the polygonal model

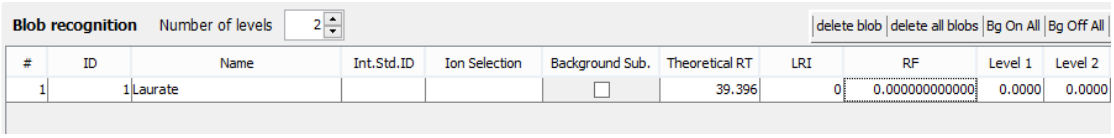


Figure 6.5: The Method Panel with the new Blob ID

6.2.4.3 Define ROI

The menu item command is used to manually define the selected region as a *Region of Interest*(ROI). The ROI description is maintained inside the Method and is displayed in the Method Panel.

After this operation is possible to integrate only the Region of Interest (see Chapter 9) instead of the full chromatogram.

6.2.4.4 Define noise/min. area

The menu item command is used to manually define the selected region as a *background region* for the purpose of defining the Method parameters *noise* and *minimum* area [2] (see Section 7.2.2).

These parameters are initially defined by default as -1 (meaning that the software automatically calculates an optimal estimate over the whole chromatogram) and may be changed by the user in order to obtain a better integration; in many cases, however, the user does not know which values to assign.

The menu item `Define noise/min. Area` can be used for this purpose. The user should select a rectangular region that can be considered as *background* (that is a region which does not contain significant Blobs) and then give this command.

The software calculates an estimate of ASTM noise and minimum area throughout the selected area, and automatically assign them to the current Method. The user can then watch the values by switching to the Method Panel, and finally perform a new integration to apply these settings.

The operation may be obviously repeated more times on different regions, until a satisfying pair of value has been obtained.

[2] The minimum area parameter is very important: values too large have the effect of hiding significant Blobs, whereas values too small may cause the recognition of thousands of small Blobs, which is a time-consuming activity which can last for minutes.

6.2.4.5 Edit

This menu item command opens a Local Chromatogram Editor window. See 11.10 for a full description.

This feature is reserved for an advanced use of the software. The menu command may be hidden or shown according to a special configuration option (please contact Technical Support for managing this option).

6.2.4.6 Cancel

The menu item cancel the context menu without performing any operation.

6.2.5 Map View Point Context Menu

This context menu is displayed after a single click with the right mouse button on a point of the Map View. It contains three kinds of items:

- items for advanced editing of the Blobs;
- items for advanced zoom operations.
- items for advanced printing operations.

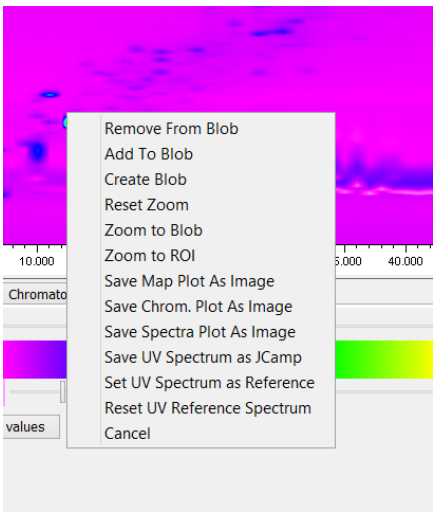


Figure 6.6: The Map View Point Context Menu

The items are described in the following Sections.

6.2.5.1 Remove From Blob

If the mouse is placed on a point of the Map View which is located *inside* a Blob, this will be deleted from the current Blob list.

6.2.5.2 Add To Blob

If the mouse is placed on a point of the Map View which is located *inside a Peak belonging to a modulation which is adjacent to a Blob*, the Peak will be added to the Blob.

6.2.5.3 Create Blob

If the mouse is placed on a point of the Map View which is located *inside a Peak not belonging to any Blob*, a new Blobwill be created, containing only that Peak.

6.2.5.4 Reset Zoom

The command cancels the current zoom and restores the initial Map View, showing the full Chromatogram.

6.2.5.5 Zoom to Blob

The command sets the current Zoom in such a way to show the currently selected Blobat a maximum enlargement, filling the whole Map View.

6.2.5.6 Zoom to ROI

The command sets the current Zoom in such a way to show the current ROI at a maximum enlargement, filling the whole Map View.

6.2.5.7 Save Map Plot as Image

This command and the next two ones allow the user to save various images without moving the mouse cursor from the currently selected point. The commands execute the same action implemented by the main Menu command `File|Save Map as Image` described at 4.1.7 (that is, opening a window for the choice of the image file, fonts, sizes, etc.): the Main menu commands, however, mean that the user should

move the mouse to the Main menu position, losing the current position in the Map View. With the context menu commands the current position is maintained, and it is also possible to generate different types of images (not only Map Plot, but also Chromatogram Plot and Spectra Plot).

6.2.5.8 Save Chromatogram Plot as Image

See 6.2.5.7.

6.2.5.9 Save Spectra Plot as Image

See 6.2.5.7.

6.2.5.10 Save UV Spectrum as JCamp

This command and the next two apply only to datafiles containing UV spectra. The command is used for saving the *Current Spectrum* as a JCamp file (the current spectrum is the spectrum corresponding to the current mouse position in the Map View, which is displayed in the UV Spectrum window).

The JCamp file is saved in the same folder of the datafile, with a name made up by the name of the datafile, followed by the Retention Time of the point and by the extension "`.jdx`".

The software saves these files without generating informative or confirmation messages: in this way the user can save more spectra without interruptions.

A Jcamp file may be subsequently loaded as *Reference Spectrum* which can be used for spectra comparison and similarity calculation.

6.2.5.11 Set UV Spectrum as Reference

The command is used for setting the *Current Spectrum* (that is the spectrum corresponding to the current mouse position in the Map View) as *Reference Spectrum* to be used for spectra comparison and similarity calculation.

As soon as the Reference Spectrum is defined, the Spectra Plot will show the Reference Spectrum (*blue* line) together with the Current Spectrum (*black* line).

This command allows an easy definition of the Reference Spectrum, avoiding the passage through the JCamp file.

The command may be used repeatedly for easily testing different spectra as reference.

6.2.5.12 Reset UV Reference Spectrum

The command allows to *reset* the Reference Spectrum, in order to show in the Spectra Plot only the Current Spectrum.

In fact, showing a Reference Spectrum can be misleading when this is no longer used.

6.2.5.13 Cancel

The command clears the Map View Point Context Menu in the cases when this is not automatically cleared upon completion of the selected operation.

6.3 The Graphical Parameters page

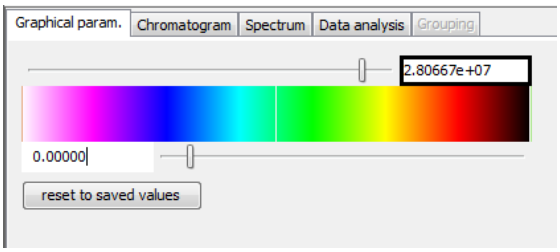


Figure 6.7: Graphical Parameters Page

This panel contains some controls designed to define with precision the *Color Mapping* that the software uses to represent the Chromatogram Map (Contour Plot) described in Section 6.2.

Color Mapping is the rule which transforms (*maps*) an *absorbance* or *intensity* value into a pixel *color* value. Color Mapping is defined by assigning:

- the *Color Map*;
- the minimum and the maximum of the *intensity* scale;
- the minimum and the maximum of the *color* scale.

The panel is shown in the Figure 6.7. It contains:

- an upper pair of controls, including a slider and a text field; these controls are used to define the *maximum* of the *intensity scale*, as described in 6.3.2;
- an intermediate colored bar, which represents the color scale;
- a lower pair of controls, including a slider and a text field; these controls are used to define the *minimum* of the intensity scale, as described in 6.3.2;
- a *Reset* button; this allow to restore the previously saved values of all scales.

6.3.1 The Color Map

The term "Color Map" refers to a set of instructions internally defined in the software which implements the transformation between *intensity values* and *color values*, that is between an *intensity scale* and a *color scale*.

Only one Color Map is presently defined in the software; this is a classical Color Map, frequently used in this types of representations; it scans from White to Black, passing through a series of colors (Magenta, Cyan, Blue, Green, Yellow, Orange, Red, Brown).

6.3.2 The intensity scale

The minimum and the maximum values of the intensity scale are initially calculated by the software and can then be modified by the user in order to highlight some details instead of others.

The software calculates the initial (default) values of the intensity scale as the mathematical minimum and the maximum of the intensity values of the *whole* chromatogram.

Minimum and maximum of the intensity scale can be modified through the two *slider* controls (upper and lower) of the Graphical Parameters Window. The upper control defines the maximum, the lower control defines the minimum.

The user can manually change these values acting in two ways:

- directly writing the numerical values in the *text fields*;
- scrolling the corresponding *sliders*.

6.3.3 The color scale

In the middle, between the two slider controls, there is an horizontal *colored bar*, which represents the *color scale*.

The minimum and the maximum of the color scale are shown as little *vertical white lines* inside the colored bar (the left line obviously representing the minimum, and the right line the maximum).

The user can manually change the minimum and/or the maximum values of the color scale in two ways:

- dragging with the mouse the corresponding vertical white line towards left or towards right;
- clicking with the mouse near one of the vertical white lines: the line will move automatically to the current mouse position.

6.4 The Chromatogram page

The chromatogram page shows the *linear chromatogram*, that is the plot of *intensity* vs. *modulation time*, for each *modulation*.

The chromatogram displayed in the Chromatogram page represents the modulation corresponding to the mouse pointer position in the main Map View.

As the user moves the mouse in the upper window, the plot quickly changes in the lower window, following the various modulations: the plot changes so much faster as modulations are "dense" in the main plot. Use the zoom for a less dense representation of modulations, allowing a more precise identification of a single modulation.

The plots of the Chromatogram page shows two *cursors*, that is vertical lines of different colors:

The *blue* cursor
: is the current position of the mouse in the Map View.

The *red* cursor
: is the current position of the mouse in the Chromatogram View.

The linear chromatogram may be shown in 4 different ways, activated by corresponding buttons placed at the top of the chromatogram page (see Figure 6.8).

A fifth button displays a pop-up dialog which allows the user to set the intensity scale.

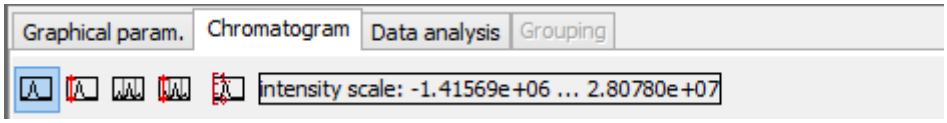


Figure 6.8: The Toolbar of the Chromatogram page

6.4.1 Single modulation in zoom interval

This representation shows the plot of the chromatogram concerning the *current modulation* (that is the modulation corresponding to the current mouse pointer position in the 2D Map View), on a time domain that is a *fraction* of the *modulation time*, corresponding to the vertical zoom interval in the 2D Map View

More precisely:

- Abscissa axis is *retention time*, ranging within the limits of modulation time set by the current zoom area (that is between the lower and upper limits of the zoom rectangle).
- Ordinate axis is *intensity*, ranging from 0 to an upper limit corresponding to the maximum intensity. The user can change the limit through the control `Max. Int.`, directly writing a new value in the text field.

See Figure 6.9.

6.4.2 Single modulation in whole modulation interval

This representation shows the plot of the chromatogram concerning the *current modulation* (that is the modulation corresponding to the current mouse pointer position in the 2D Map View), on a time domain corresponding to the *whole modulation time*.

More precisely:

- Abscissa axis is *retention time*, ranging from o to the modulation time.
- Ordinate axis is *intensity*, ranging from 0 to an upper limit corresponding to the maximum intensity. The limits can be changed as described in Section 6.4.1.

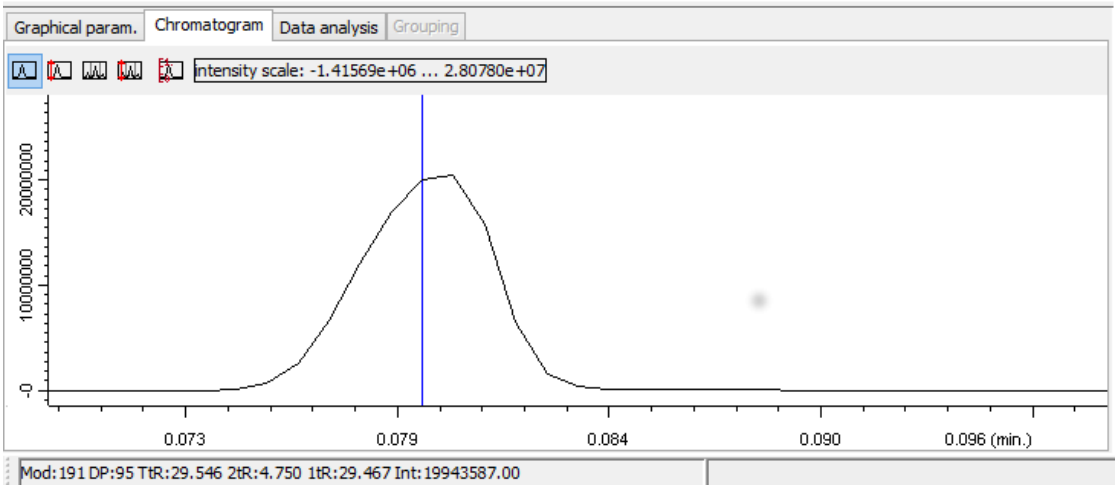


Figure 6.9: Single modulation in zoom interval

See Figure 6.10.

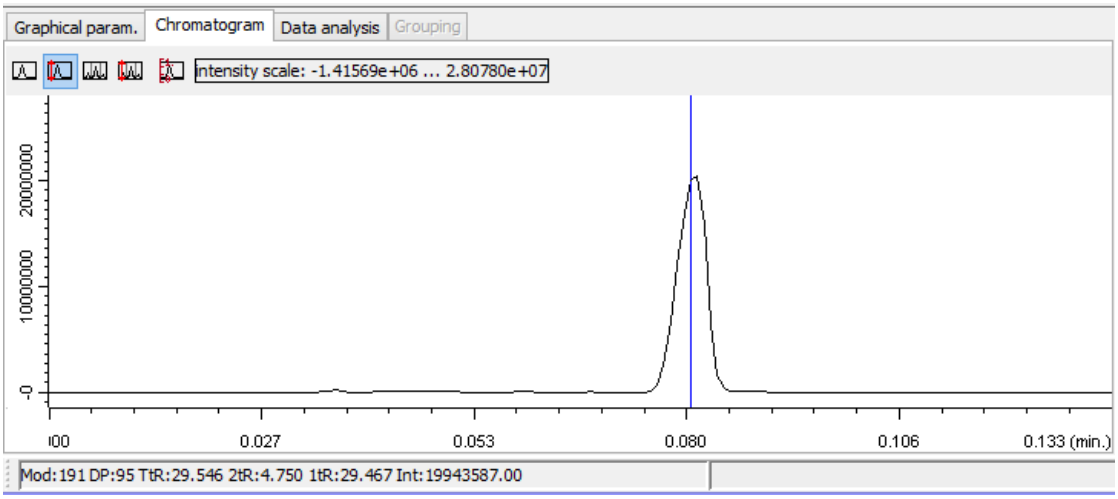


Figure 6.10: Single modulation in whole modulation interval

6.4.3 Multiple modulation in zoom interval

This representation shows the plot of all chromatograms concerning the *modulations included in the current horizontal zoom interval*, on a time domain that is a *fraction* of the *modulation time*, corresponding to the vertical zoom interval in the 2D Map View.

More precisely:

- Abscissa axis is *retention time*, ranging within the limits of modulation time set by the current zoom area (that is between the lower and upper limits of the zoom rectangle).
- Ordinate axis is *intensity*, ranging from 0 to an upper limit corresponding to the maximum intensity. The limits can be changed as described in Section 6.4.1.

See Figure 6.11.

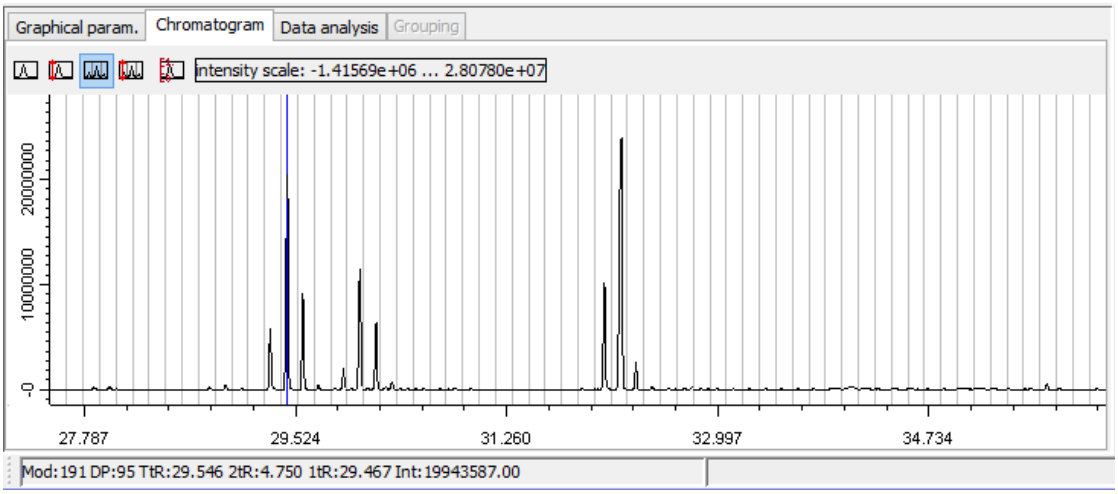


Figure 6.11: Multiple modulation in zoom interval

6.4.4 Multiple modulation in whole modulation interval

This representation shows the plot of all chromatograms concerning the *modulations included in the current horizontal zoom interval*, on a time domain corresponding to the *whole modulation time*.

See Figure 6.12.

More precisely:

- Abscissa axis is *retention time*, ranging within the limits of modulation time set by the current zoom area (that is between the lower and upper limits of the zoom rectangle).

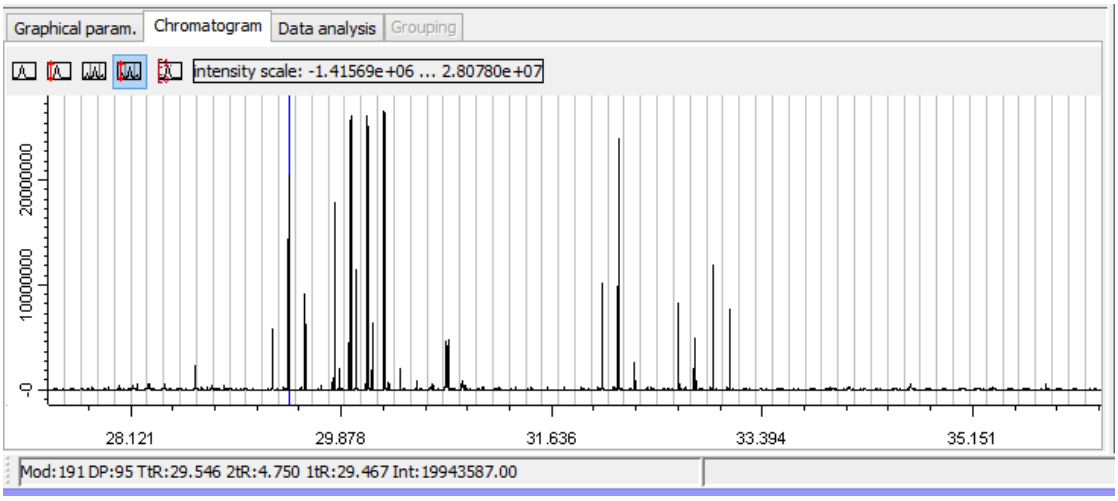


Figure 6.12: Multiple modulation in whole modulation interval

- Ordinate axis is *intensity*, ranging from 0 to an upper limit corresponding to the maximum intensity. The limits can be changed as described in Section 6.4.1.

6.4.5 Set Intensity Scale

The dialog box allows the user to define the minimum and maximum values of the intensity scale of the Chromatogram plots, as shown in Figure 6.13.

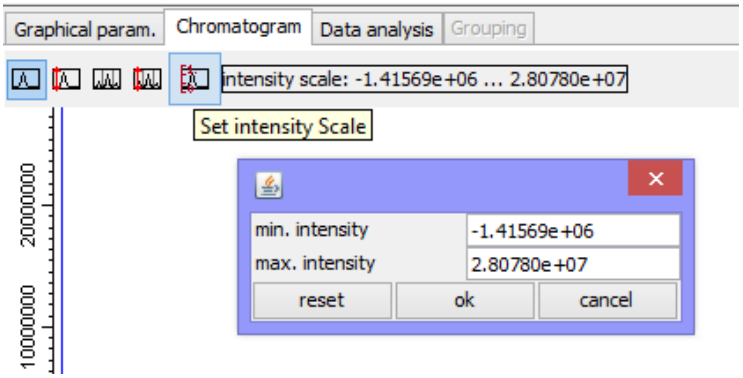


Figure 6.13: Setting Intensity Scale Dialog Box

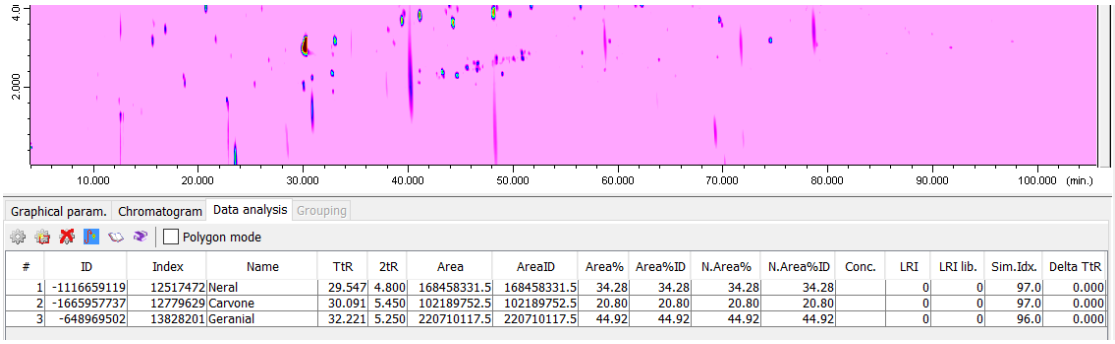
6.5 The Data Analysis page

The Data Analysis page shows the results of the integration and identification activities.

The Data Analysis page is characterized by:

- The *Data Analysis Table*; table *rows* correspond to the Blobs, table *columns* report the characteristics of each Blob.
- The *Data Analysis tool bar*: it contains some buttons used to easily perform the most common operations.
- The *Data Analysis context menu*: it applies to the currently selected Blob, and offers options for further investigation of its properties.

An example of the Data Analysis page is reported in Figure 6.14. Since the table is rather large, the Figures 6.15 and 6.16 report an enlarged view for a better readability.



#	ID	Index	Name	TtR	2tR	Area	AreaID	Area%	Area%ID	N.Area%	N.Area%ID	Conc.	LRI	LRI lib.	Sim.Idx.	Delta TtR
1	-1116659119	12517472	Neral	29.547	4.800	168458331.5	168458331.5	34.28	34.28	34.28	34.28		0	0	97.0	0.000
2	-1665957737	12779629	Carvone	30.091	5.450	102189752.5	102189752.5	20.80	20.80	20.80	20.80		0	0	97.0	0.000
3	-648969502	13828201	Geranial	32.221	5.250	220710117.5	220710117.5	44.92	44.92	44.92	44.92		0	0	96.0	0.000

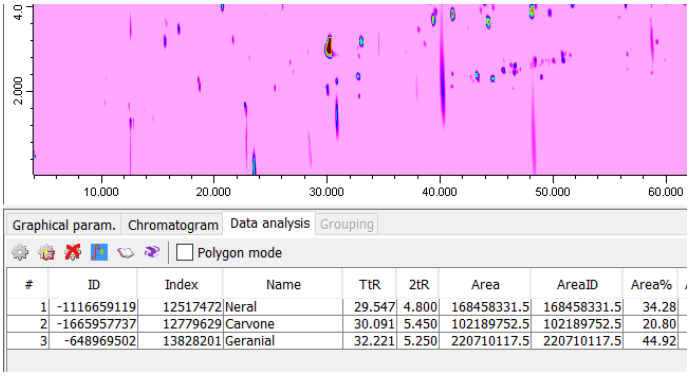
Figure 6.14: Data Analysis page - Overall view

6.5.1 The Data Analysis table

The table has a vertical scrolling bar, since the number of Blobs can exceed the available space.

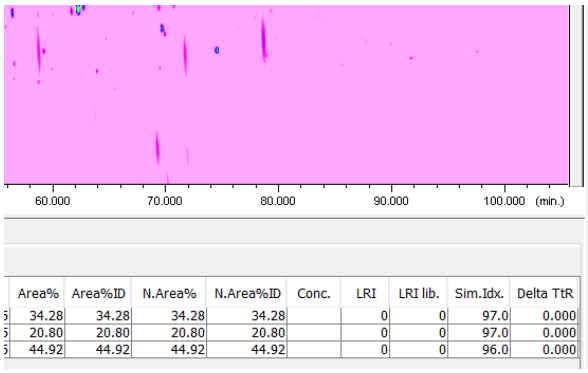
The different columns have the following meaning[3]:

[3] According to the type of analysis, some columns may be empty. For example, if Quantitative Analysis has not yet been done, the Concentration column is void.



#	ID	Index	Name	TtR	2tR	Area	AreaID	Area%
1	-1116659119	12517472	Neral	29.547	4.800	168458331.5	168458331.5	34.28
2	-1665957737	12779629	Carvone	30.091	5.450	102189752.5	102189752.5	20.80
3	-648969502	13828201	Geranial	32.221	5.250	220710117.5	220710117.5	44.92

Figure 6.15: Data Analysis page - Left view



Area%	Area%ID	N.Area%	N.Area%ID	Conc.	LRI	LRI lib.	Sim.Idx.	Delta TtR
34.28	34.28	34.28	34.28		0	0	97.0	0.000
20.80	20.80	20.80	20.80		0	0	97.0	0.000
44.92	44.92	44.92	44.92		0	0	96.0	0.000

Figure 6.16: Data Analysis page - Right view

1. #**(Count)**. This is just a *row counter*, an easy reference for the large tables; it is not related to the Blob, since the ordering may change.

2. **Index**. The Index is an *integer number* that unambiguously identify the Blob, intended as a *geometrical entity*; see Section 3.1.5 for a description of this and other quantities.

3. **ID**. The Blob ID, see 3.1.6.

4. **Name**. Name of the Blob.

5. **TtR**. Total retention time of the Blob.

6. **2tR**. Retention time in 2nd dimension of the Blob.

7. **Area**. Area of the "individual" Blob.

8. **Area ID**. Sum of the areas of all Blobs having the same ID. In the case of Blobs Grouping, this is the overall area of the Group of Blobs: it is the same for all the Blobs belonging to the Group.

9. **Area %**. Area percent of the individual Blob, calculated with respect to the sum of the areas of all Blobs.

10. **Area % ID**. Sum of the areas percent of all Blobs having the same ID. In the case of Blobs Grouping, this is the overall area percent of the Group of Blobs.

11. **Normalized Area %**. Area percent of the individual Blob, normalized with respect to the sum of the areas percent of all *identified* Blobs.

12. **Normalized Area % ID**. Sum of the areas percent of all Blobs having the same ID, normalized with the respect to the sum of the areas percent of all *identified* Blobs.

13. **Concentration**. This column will be filled after quantitation.

14. **LRI**. The Linear Retention Index of the Blob, calculated by the software against the reference table.

15. **LRI lib**. The Linear Retention Index of the most similar compound in the LRI library.

16. **Sim. Index**. The Similarity Index of the LRI calculated with respect to the LRI from the library.

17. **Delta TtR**. The Time Shift between the Total Retention Time of the Blob and the Total Retention Time of the identified compound stored in the Method. The Time Shift is calculated as the absolute value of the difference between the two times, normalized with respect to the Method value, and expressed as a *percentage*,

6.5.1.1 A Grouping sample

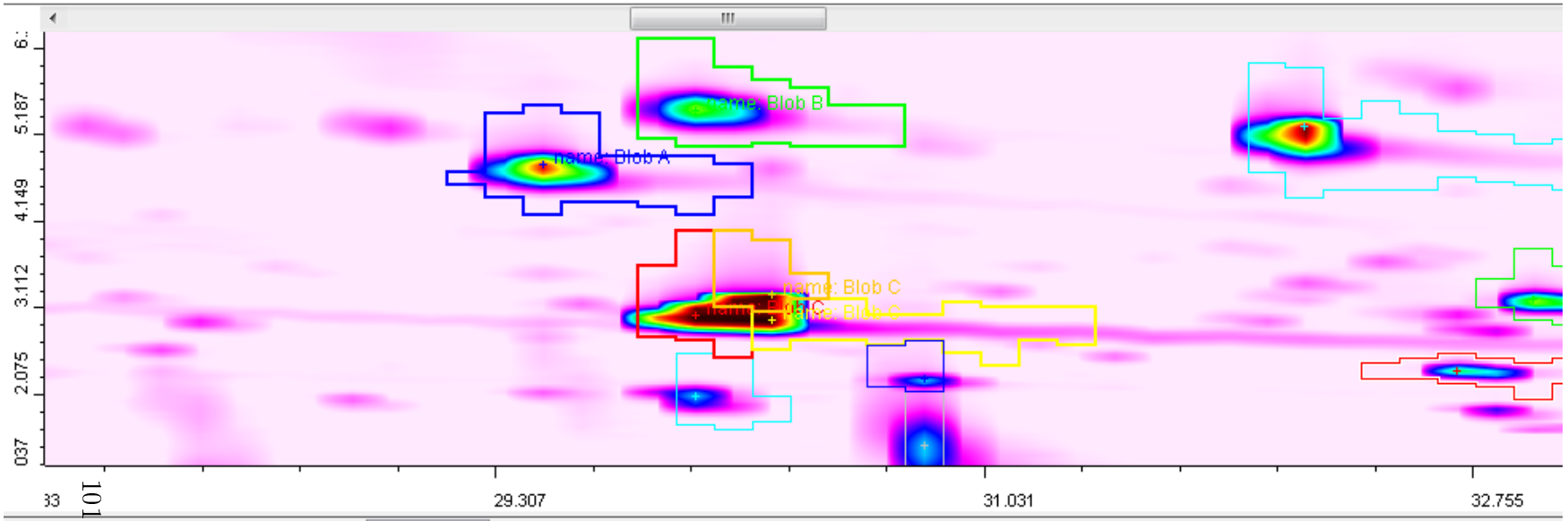
The Figure 6.17 shows an example af Data Analysis table for the ID Grouping case.

In the sample shown, three Blob IDs have been manually assigned: Blob A and Blob B are relative to individual Blobs, whereas Blob C is relative to an area which include 3 different individual Blobs.

As can be seen, the first element that catches the eye is that the Blobs which belong to a Group are represented with a *grey* background.

Examining these 3 Blobs we can see that:

- The quantity called `Area ID` is the sum of the `Area` of the 3 Blobs and is equals for all them.
- The quantity called `Area %` is the fraction of the Blob Area with respect to the Area of *all* Blobs: the sum of all these values is then 100.
- The quantity called `Area % ID` is the sum of the `Area%` of all Blobs having the same ID. Whereas for Blob A and Blob B this corresponds to the `Area %`, in the case of Blob C this corresponds to the sum of 3 Blobs and it is equal for all them (22.55 + 8.25 + 26.80 = 57.60). The sum of all `Area % ID` must obviously take into account just one value for each Blob ID (10.77, 6.51, 57.60); this sum is less then 100 because there are unidentified Blobs.
- The quantity called `Normalized Area %` is the fraction of the `Area` of the Blob with respect to the Area of *all identified* Blobs; the sum of all these value is 100.
- The quantity called `Normalized Area % ID` is the fraction of the `Area % ID` of the Blob with respect to the sum of the `Area % ID` of *all identified* Blobs; the sum of all `Normalized Area % ID` must obviously take into account just one value for each Blob ID; the sum of all these value is 100.



Graphical param. | Chromatogram | Spectrum | Data analysis | Grouping

Polygon mode

Index	ID	Name	TtR	2tR	Area	Area ID	Area%	Area% ID	Norm.Area%	Norm.Area% ID	Conc.
12517472	1	Blob A	29.547	4.830	169894187.8	169894187.8	10.771	10.771	14.384	14.384	
12779561		unknown	30.034	2.063	18584632.0	0.0	1.178	0.000	0.000	0.000	
12779580	3	Blob C	30.050	3.019	355693441.2	908529232.1	22.551	57.601	30.114	76.918	
12779629	2	Blob B	30.091	5.484	102736439.8	102736439.8	6.514	6.514	8.698	8.698	
12910651	3	Blob C	30.316	2.969	130120473.8	908529232.1	8.250	57.601	11.016	76.918	
12910657	3	Blob C	30.321	3.270	422715317.1	908529232.1	26.800	57.601	35.788	76.918	
13172765		unknown	30.824	1.459	50743184.4	0.0	3.217	0.000	0.000	0.000	
13172781		unknown	30.838	2.264	10440173.4	0.0	0.662	0.000	0.000	0.000	
13828201		unknown	32.221	5.283	222768001.3	0.0	14.124	0.000	0.000	0.000	
14090287		unknown	32.706	2.365	26301474.0	0.0	1.668	0.000	0.000	0.000	
14221376		unknown	32.987	3.220	56763611.5	0.0	3.599	0.000	0.000	0.000	
15007806		unknown	34.585	3.119	10517136.5	0.0	0.667	0.000	0.000	0.000	

Figure 6.17: Data Analysis page - Example showing ID Grouping case

6.5.2 Customizing the Data Analysis Table

The Data Analysis Table contains the most important results of $Chrom^{square}$ analysis: for this reason, a set of options have been provided for the user, in order to customize the table according to special needs or user preferences. he Columns

6.5.2.1 Customizing the Positions of the Columns

The original ordering of the columns is shown at section 6.5.1. The user may however change this ordering at any time.

To change the position of a column:

1. Click with the left mouse button on the header of the column.
2. *Drag* the column to the new position (that is, move the mouse while holding down the mouse button.
3. Release the mouse button when the column is in the desired new position.

The operation may be repeated for all columns.

The assigned positions are saved automatically, so that the new disposition is maintained when changing page or restarting the software.

The original default ordering may however be easily restored through a menu command. See section 6.5.2.4.

6.5.2.2 Customizing the Widths of the Columns

The original widths of the columns are assigned by the software, which will try to show the whole contents of all columns. In this way, however, the total width of all columns normally exceeds the available table space, so that the user should use the horizontal scroll bar.

The user may however change theses widths at any time.

To change the widths of a column:

1. Click with the left mouse button on the separator between the headers of two columns.

2. *Drag* the separator to a new position (that is, move the mouse while holding down the mouse button). This operation changes the width of the *left* column.

3. Release the mouse button when the column width is satisfactory.

The operation may be repeated for all columns.

The assigned widths are saved automatically, so that they are maintained when changing page or restarting the software.

The original default widths may however be easily restored through a menu command. See section 6.5.2.4.

6.5.2.3 Showing all Columns

In some case, may be useful to see all columns at a glance, without resizing or scrolling.

To show all columns:

- Click with the right mouse button on the Header of the Table.
- From the context menu that will appear, select `Show All Columns`.

The command must be used with care, since it destroys the manually assigned column widths.

The values of the new columns widths are set proportional to the original default widths.

6.5.2.4 Restoring Default Positions and Widths of the Columns

This command is used to restore the original default widths and positions: this may be useful when, after too many manual changes, the user wants to restart from a clean configuration.

To restore the default values:

- Click with the right mouse button on the Header of the Table.
- From the context menu that will appear, select `Reset All`.

6.5.2.5 Sorting the Columns

The Blobs Table may be sorted according to the values of any column, both in ascending order and in descending order.

To sort according a column:

- Double-click with the left mouse button on the header of the column.
- The first time the Table is sorted in ascending order.
- Double-click again to sort in descending order.
- At each double-click the order is inverted again.

6.5.3 The Data Analysis Toolbar

The Toolbar is shown in Figure 6.18.

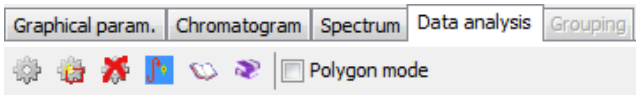


Figure 6.18: Data Analysis page - The Data Analysis Toolbar

It contains a sequence of buttons; most buttons are *shortcuts* for commands that can be also executed through menu commands; the last button perform a specific operation.

The buttons are:

1. (*gear*) **Integrates the whole chromatogram**. It corresponds to the menu Action command `Integrate Whole Chromatogram` of Section 4.6.2.
2. (*gear with red rectangle*) **Integrates only the defined Regions of Interest**. It corresponds to the menu Action command `Integrate ROI` of Section 4.6.1.
3. *(gear with a red delete cross*) **Removes integration**. It corresponds to the menu Action command `Remove integration` of Section 4.6.4.
4. (*wrap "S" red sign on a blue background*) **Correct wrap around**. It corresponds to the menu Action command `Correct wrap around` of Section 4.6.15.

5. (*book*) **Search selected Blob** in spectra library. It corresponds to the menu Action command `Search selected Blob` of Section 4.6.13.

6. *(book collection)* **Search all Blobs** in spectra library. It corresponds to the menu Action command `Search all Blobs` of Section 4.6.14.

7. (*check button*) **Polygon mode**. This is a switch which controls the mode of defining the Blob regions on the 2D Plot: if unchecked, the normal mode is active (rectangular selection, see Section 6.2.3 and 6.2.4.2); if checked, the Polygon mode is active (see Section 11.4)

6.5.4 The Data Analysis Context Menu

To show the Data Analysis context menu the user must:

- select a Blob, by clicking with the *left mouse button* on the corresponding row: the selected row will be shown with a *blue* background;
- click with the *right mouse button* on the selected row.

Note that the Blob corresponding to the selected row will be also shown in the 2D Map View with *enhanced boundaries*, like in Figure 6.19.

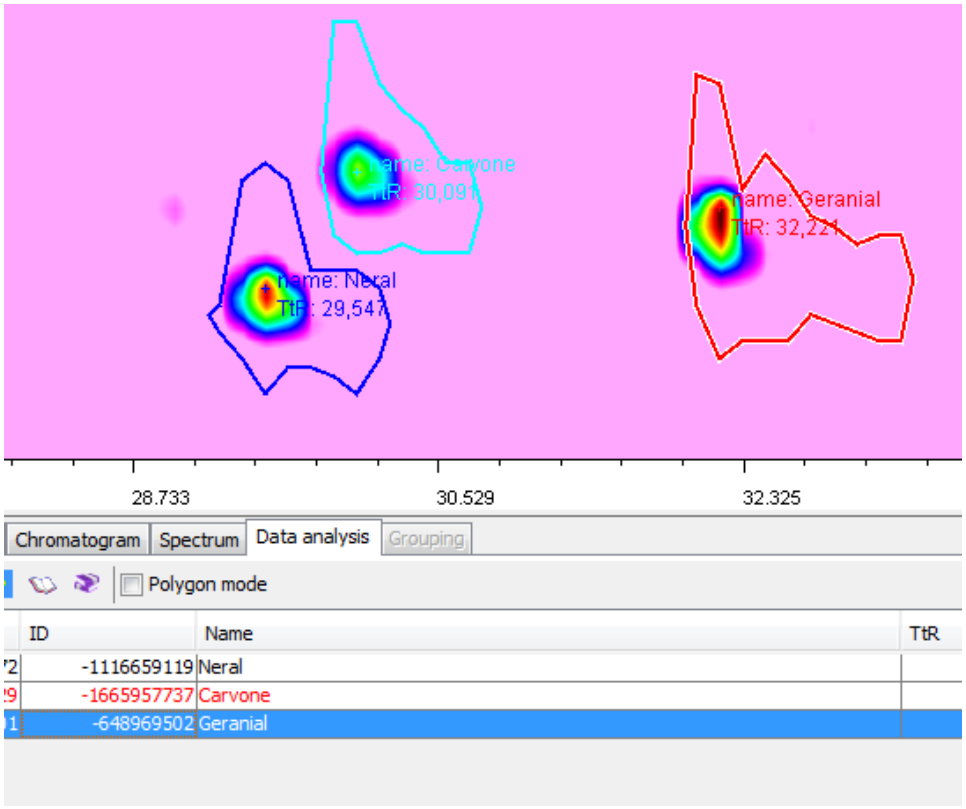


Figure 6.19: Enhanced representation of the selected Blob

Normally the user should select only one Blob at a time, since most menu functions are meaningful just for one Blob, except for the case of the first menu item, Search Selectected Blobs: in this particular case the multiple selection (both of contiguous and not-contiguous rows) can be applied. For all other menu items the multiple selection is not allowed: giving a menu command while more than one row are selected will have no effect.

The context menu is represented in Figure 6.20. The various items are described in the following sections.

6.5.4.1 Search Selected Blobs

This menu item is implemented for the license types involving the *automatic Blob Search* (see Section 8.3.2).

This is the only menu item which allows the *multiple selection* of the table rows. The usual rules of multiple selction apply:

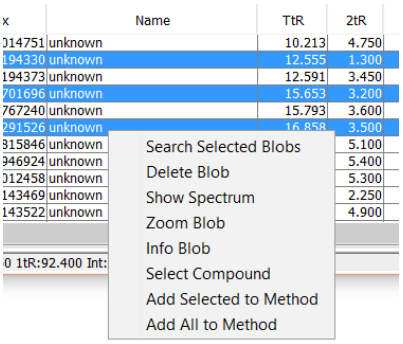


Figure 6.20: Data Analysis page - The Data Analysis Context Menu

- To select a *range of contiguous rows*, click on the first and on the last row of the range holding down the *SHIFT* key.
- To select more rows (contiguous or not contiguous), click on each row holding down the *CONTROL* key.

The command executes a search of the currently selected Blob(s) using the external software component.

If the search is successful, a Name and a Blob ID are assigned to the Blob(s), and immediately shown both on the 2D Map View and in the Data Analysis table.

6.5.4.2 Delete Blob

The command deletes the currently selected Blob from the list of the integrated Blobs.

The 2D Map View and the Data Analysis table are updated as well.

6.5.4.3 Show Spectrum

This menu item is implemented for the license types involving the *automatic Blob Search* (see Section 8.3.2) and for chromatograms which contain Mass Spectra.

In these cases, the command shows a new window, called *Spectrum Viewer*, which displays the *reference* Mass Spectra of the *currently selected Blob*. These spectra are different from the ones shown in the Spectra View, that are the spectra relative to the current position of the mouse pointer on the Plot View.

A sample of Spectrum Viewer window is given in Figure 6.21.

The window contains two *panels*, each panel displaying two spectra plots.

1. The upper panel displays the spectra plots with the intensities (vertical scale) represented as *absolute* values.
2. The lower panel displays the spectra plots with the intensities (vertical scale) represented as *normalized* values (that is values ranging from 0 to 100).

Each panel shows two spectra plots:

1. The *Blue* plot is the spectrum of the currently selected Blob;
2. The *Green* plot is the spectrum of the currently selected Blob with *background subtracted* (if this option is selected in the Method).

Each plot represents the spectrum according to the usual practice, that is m/z along the abscissa axis, and *intensity* along the ordinate axis.

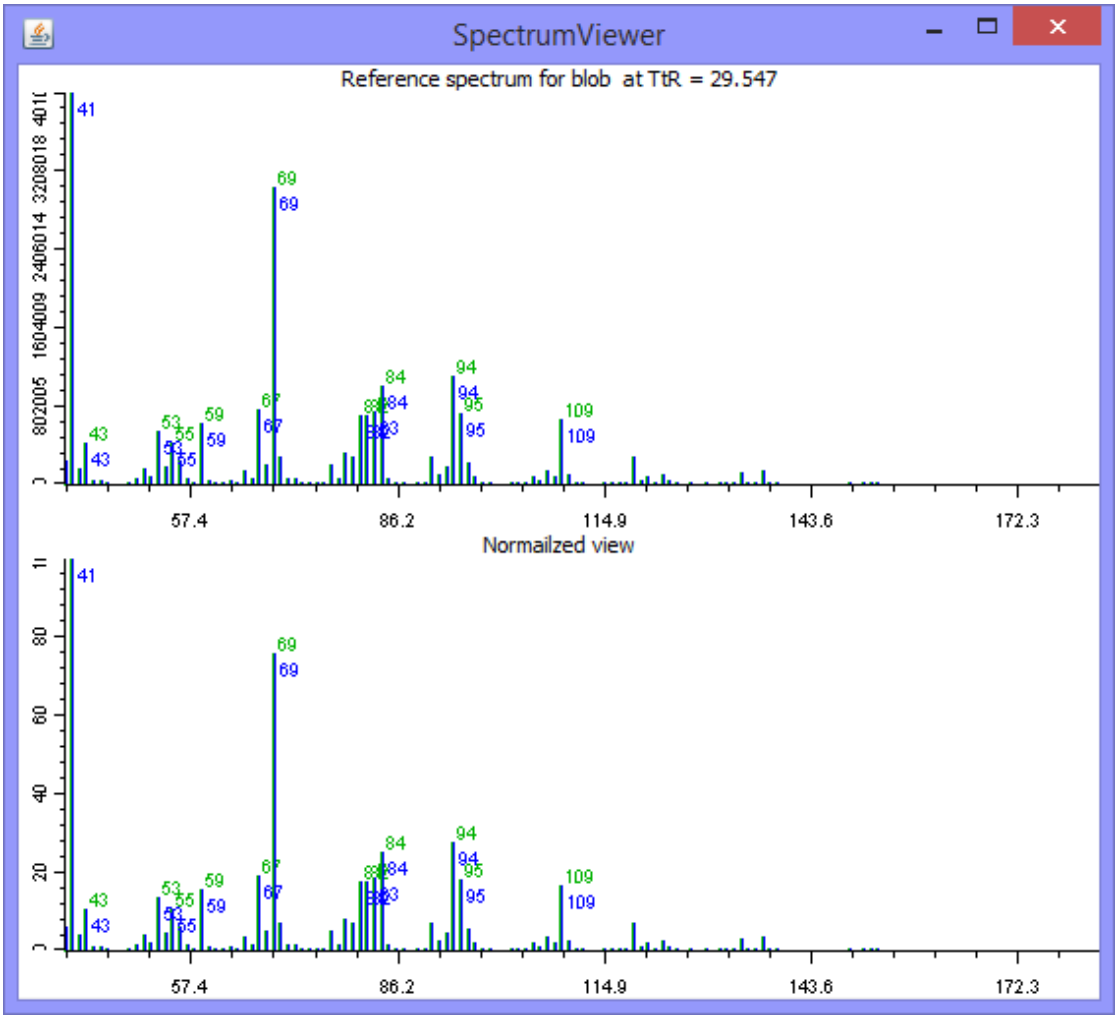


Figure 6.21: Spectrum Viewer window

6.5.4.4 Zoom Blob

The command automatically performs a zoom of the 2D Plot Map in such a way that the currently selected Blob is shown at the maximum enlargement, filling the whole available space of the window. A sample can be seen in Figure 6.22.

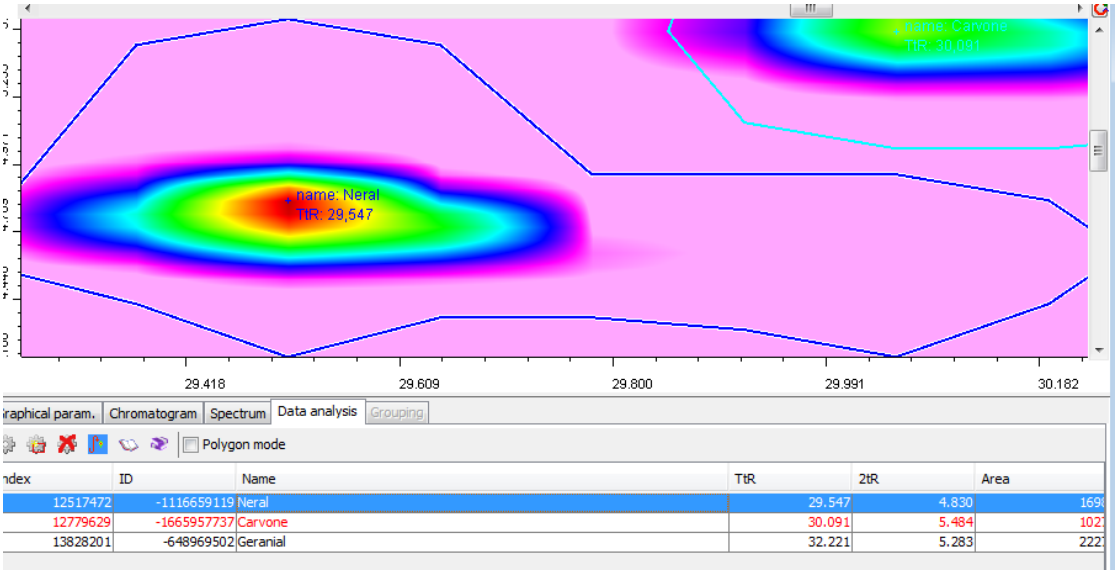


Figure 6.22: A full zoomed Blob

6.5.4.5 Info Blob

The command displays a window which reports a description of the Blob, including:

- general information about the Blob;
- a list of all peaks corresponding to the various modulations, with a very detailed description for each of them;
- detailed information about the spectra at the top point of the Blob: this information can be particularly useful for the fine tuning of MRM spectra analysis.

A sample is shown in Figure 6.23.

This window may be useful in special cases, when the user wishes to investigate the inner functioning of the software. It is not generally used in the daily practice.

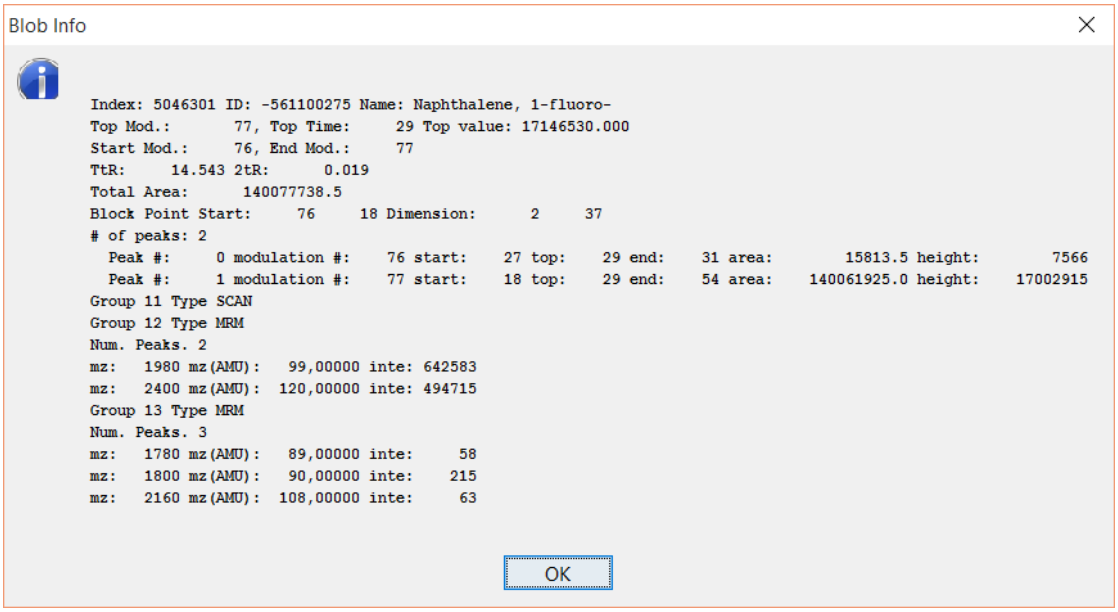


Figure 6.23: The window displaying Blob Info

6.5.4.6 Select Compound

The command allows the user to select a different compound among those returned by the *similarity search*, performed by the external software component (see Section 8.3.2)

The command is active only if the automatic Blob Search has already been performed on the selected Blob (through the context menu command `Search Blob` of Section 6.5.4.1 or the main menu command `Search All Blobs` of Section 4.6.14).

The command displays a dialog window, named `Assign compound for Blob...`, showing all compounds found by the similarity search.

The software by default identifies the Blob using the compound with the highest similarity index (the first of the list); the user can select a different compound and confirm his choice with the button `Ok`. The choice is archived, so that the assigned compound will be shown if this window will be opened at a later time (in this case, it will be however possible to change the former choice).

The dialog box is shown in Figure 6.24.

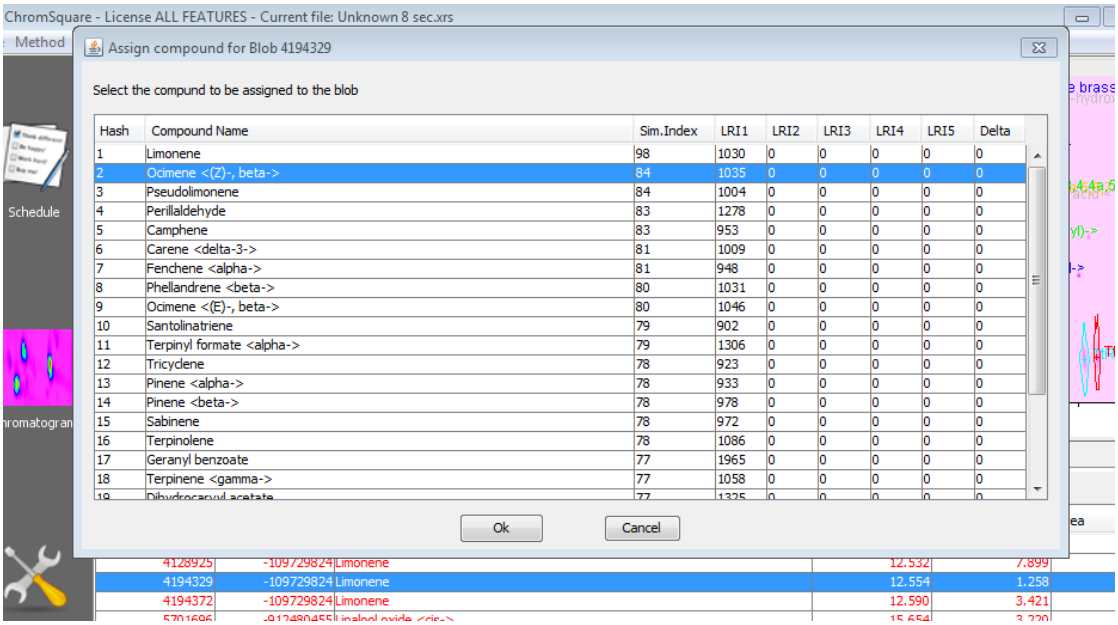


Figure 6.24: The Assign Compound dialog box

6.5.4.7 Add Selected to Method

Adds the currently *selected* and *identified* Blob to the Blob list used in the Method for quantitation (The command does not work if the current Blob has been not identified).

In fact, the identified Blobs are not automatically added to the Method, in order to avoid duplications. The user should explicitly define which Blobs are to be added to the Method, using the following procedure:

- Once the automated search for one or more Blobs has been performed, if the Blob *is not already present* in the Blob Table of the Method, the entire row is redrawn in *red* in the Data Analysis table (see Section 6.5.1).
- The user can decide to add the selected Blob to the Blob Table of the Method or not, using this command.
- As soon as this operation is performed, the Blob that has been included in the Method is available for quantitation and is redrawn in *black*.

6.5.4.8 Add All to Method

The command automatically performs the procedure of adding a Blob to the Method (described in the previous Section 6.5.4.7) on *all* the Blobs of the Blob Table.

In other words, the procedure search for all Blobs of the Table corresponding to rows marked in *red*, and add them to the Method: at the end of the procedure all rows are redrawn in black.

6.6 The Grouping page

The Grouping page is the last page of the lower window of the Chromatogram Panel. It is activated only when in the Method is defined *at least one Group* in the Group table.

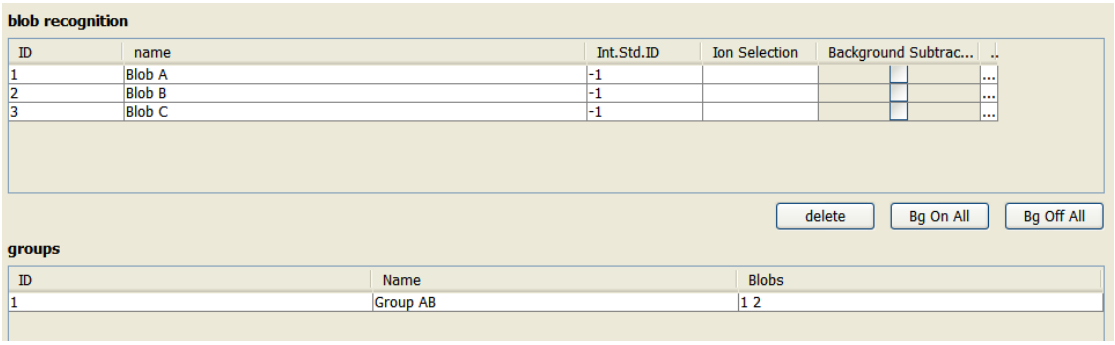


Figure 6.25: Method Panel showing one Group

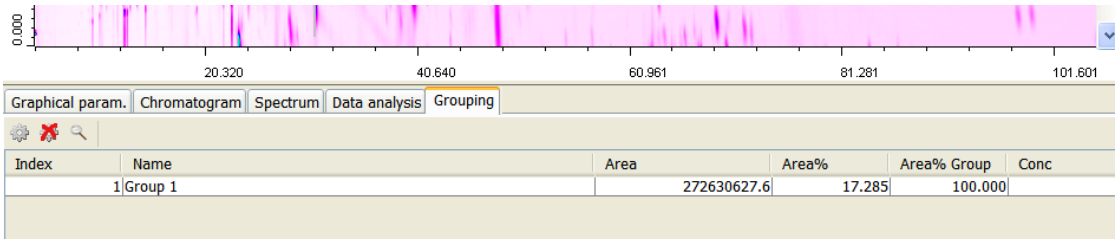


Figure 6.26: Grouping page - The results

The page consists of a table with *6 columns* and as many rows as the Groups defined in the Method. The columns gave the following meaning:

1. **Index**. This is just a counter of the rows/groups.

2. **Name**. The name assigned to the Group in the Method.

3. **Area**. The Area of the group, corresponding to the sum of the Areas of all Blobs which belongs to the Group.

4. **Area %**. The Area % of the group, corresponding to the sum of the Areas % of all Blobs which belongs to the Group.

5. **Area % Group**. The percentage of the Area % of the group, with respect to the sum of the Area % of all Groups. It obviously is 100 if only one Group is defined.

6. **Conc.**. The Concentration of the group, corresponding to the sum of the Concentrations % of all Blobs which belongs to the Group.

The Figure 6.25 shows a Method Panel with the definition of a Group. The example shown refers the same case described in Section 6.5.1.1: a Group, named "Group AB", has been defined as the aggregation of Blob A and Blob B.

The Figure 6.26 shows the results of the integration in the Grouping page. Comparing the results with those shown in Figure 6.14, it can be seen that the Area of the Group AB is equal to the sum of Areas of Blob A and Blob B. The same for Area %.

6.7 The Spectrum View

Chrom^{square} is able to display *Mass Spectra* and *UV Spectra* when these are included in the chromatographic Data File.

Mass Spectra and UV Spectra (when available) are shown in the Spectrum View, placed to the right of the Map View, which includes the two sub-windows (*tabs*) for *Mass* and *UV Spectrum View*. Only one of the two windows is active, according to the profile used to load the datafile.

As the user moves the mouse in the Map View, the spectrum quickly changes in the active Spectrum View, following the various modulations (like in the case of Chromatogram page, see 6.4).

6.7.1 The Mass Spectrum View

A sample of the Mass Spectrum View is shown in Figure 6.27.

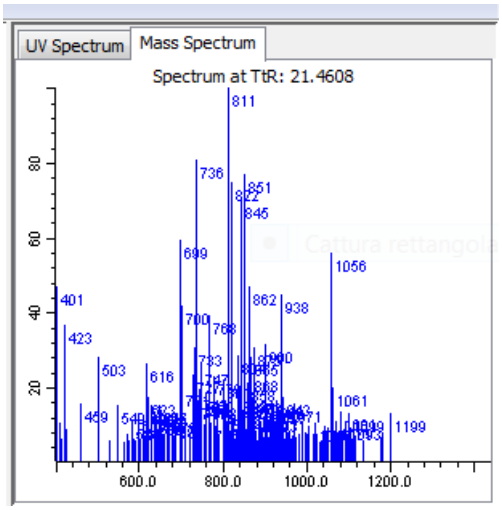


Figure 6.27: The Mass Spectrum View

The Mass spectra are represented as sets of peaks in a 2D plane, with *intensities* along the ordinate axis, and m/z along the abscissa axis.

6.7.2 The UV Spectrum View

A sample of the UV Spectrum View is shown in Figure 6.28.

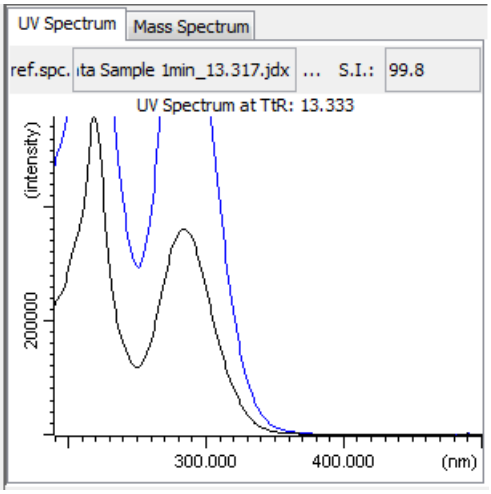


Figure 6.28: The UV Spectrum View

The UV spectra are represented as 2D plots, with *intensities* along the ordinate axis, and *wavelengths* along the abscissa axis.

The plots can contain one or two lines:

- a *black* line is always present and represents the *Current Spectrum*, that is the spectrum of the point corresponding to the current mouse position in the Plot View;
- a *blue* line represents the *Reference Spectrum* if this spectrum is currently defined. The definition of a *Reference Spectrum* can be accomplished in two ways:
  - Through the context menu command `Map View Context Menu|Save UV Spectrum as Reference`; see 6.2.5.11.
  - Loading a JCamp Reference Spectrum previously saved; see 6.2.5.10.

In the upper part of the Spectrum View there is a bar containing the following controls for the management of the reference spectrum:

- The label `ref.spc.`, meaning *Reference Spectrum*.

- A text field which displays the name of the JCamp file, if this file has been loaded as Reference Spectrum.
- A "three dots button", which can be used to select and load a JCamp file as Reference Spectrum.
- The label `S.I.` meaning *Similarity Index*.
- A text field which displays the Similarity Index calculated between the Reference Spectrum and the Current Spectrum. The Similarity Index is continuously updated as the user moves the mouse in the Map View.

6.8 The Navigator View

The *Navigator View* is a window which always contains a view of the *whole chromatogram*. When the zoom is not activated, the window shows the same image of the main Map View (even if with less details and different size). When the zoom is activated, the Navigator View window shows the chromatogram with a *rectangle* inside it, representing the *zoom area* which is displayed enlarged in the main Map View.

A sample is shown in Figure 6.29.

The window is called "Navigator" since it can be used not only for viewing the location of the zoom area, but also for *moving* or *navigating* it across the whole plot. area. The navigation can be accomplished in two different ways:

- Operating on the Navigator View;
- Operating on the *scroll bars* of the main Map View.

6.8.0.1 Moving the zoom area with the Navigator View

The user can *move* the current zoomed area operating on its representation inside the Navigator View in two ways:

- by *clicking on the center* of the new position in the Navigator View; as soon as the mouse button is released, the rectangle representing the zoom area will be moved and drawn around this new position, maintaining its previous sizes;
- by *dragging* the rectangle representing the zoom area to the new desired position.

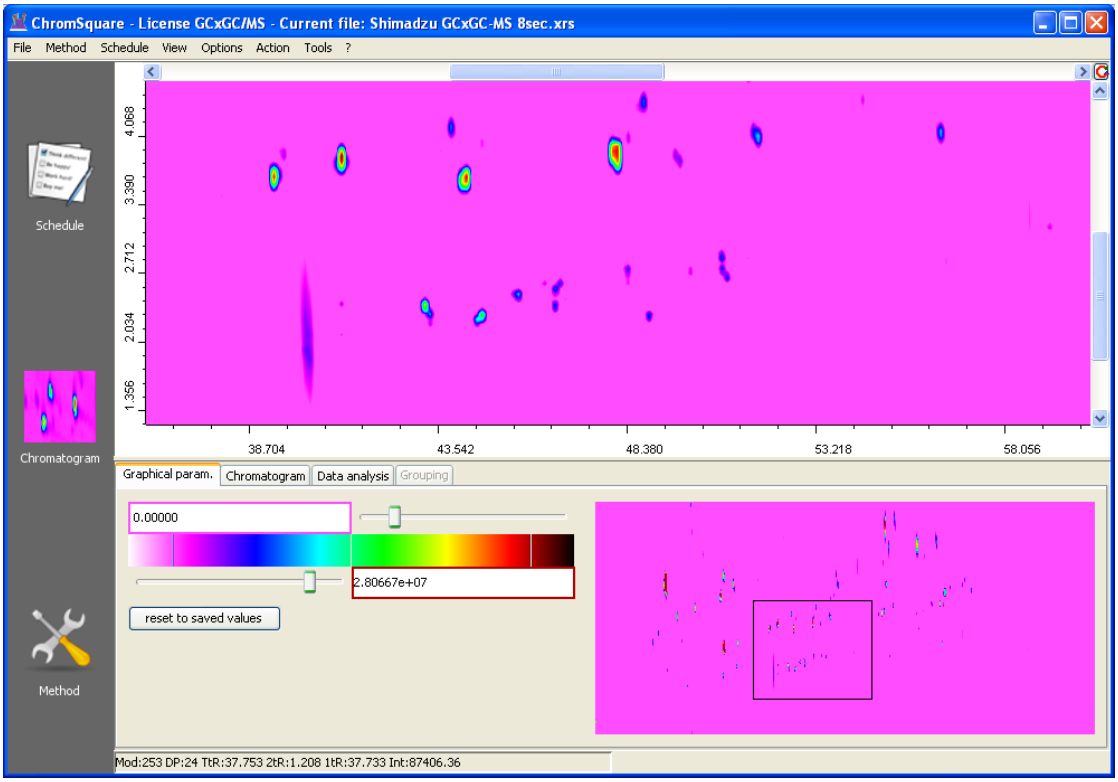


Figure 6.29: Navigator View with Zoomed Area

6.8.0.2 Moving the zoom area with the Map View sliders

The zoomed area may also be moved using the pair of *sliders* placed on the *upper side* and on the *right side* on the main Map View.

The sliders show the current *position* and *sizes* of the zoom area with respect to the overall Map View view. The fraction of the sizes of the slider with respect to the corresponding sizes of the Map View represents the fraction of the horizontal zoom area with respect to the overall chromatogram axis.

When the user moves a slider, the rectangle on the Navigator View is updated as well.

A special small button, placed in the upper right corner between the horizontal and vertical slider, can be used as a short-cut to reset the zoom (it has the same effect of the menu item `View | Reset Zoom`).

6.9 The Status Bar

The Status Bar (see Figure 6.9) shows the current position of the mouse pointer inside the Map View. It includes the following information:

- `Mod`: Modulation counter (along horizontal axis)
- `DP`: Data Point counter (along vertical axis)
- `TtR`: Total Retention Time (in minutes)
- `2tR`: Second Dimension Retention Time (in seconds)
- `1tR`: First Dimension Retention Time (in seconds)
- `Int`: Intensity (or Absorbance)

Note that: `TtR = 1tR + 2tR/60`

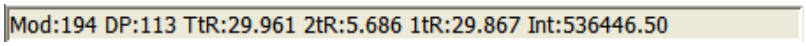


Figure 6.30: Chromatogram Panel - The Status Bar

Chapter 7

Method Panel

7.1 Panel contents

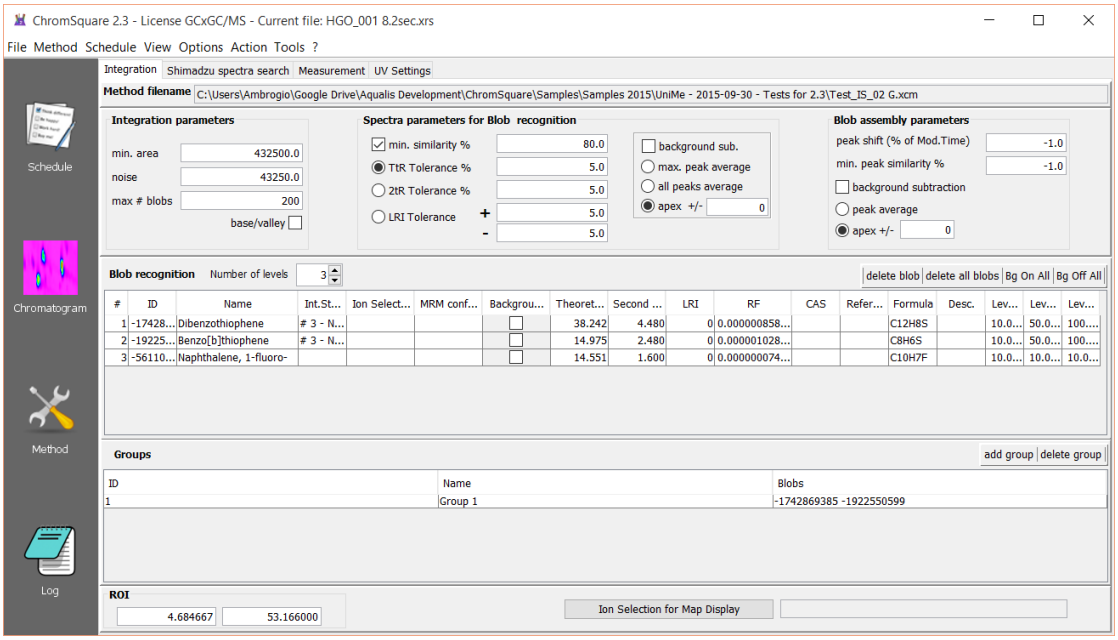


Figure 7.1: The Method Panel - Integration Page

The Method Panel is shown when the user clicks on the corresponding large icon `Method` of the Side Bar Panel (see Figure).

The Method Panel window consists of two or more pages (*tabs*): the pages `Integration` and `Measurement`are always present, whereas other pages depend on the purchased

license and on the data file type (`Shimadzu Spectra Search`, `NIST Spectra Search`, `UV Settings`).

7.2 Integration page

The Integration page is shown in Figure 7.1. Due to the large number of controls contained, the page is structured in *Panels* and *Sub-Panels*, each one regarding a particular set of parameters.

From the top to the bottom of the page we can find:

- The **Header Panel**: this just contains the file name of the Method(if already assigned).
- The **Upper Panel** or **Parameters Panel**; it contains the most part of the *scalar* parameters (that is all those common to the Methodas a whole, and not depending upon vectorial entities, like Blobs or Groups); this is divided into 3 Sub-Panels:
  - **Integration** parameters.
  - **Spectra** parameters for Blob recognition.
  - **Blob assembly** parameters.
- The **Blob ID Panel**; it contains:
  - The **Blob ID Table**.
  - The **Levels selector**.
  - The **buttons for Blob ID Table** management.
- The **Groups Panel**; it contains:
  - The **Groups Table**.
  - The **buttons for Groups Table** management.
- The **Lower Panel**; it contains:
  - The **ROI description**.
  - The **Ion selection for Map Display** button and description.

The panels and all their components are described in details in the following paragraphs.

7.2.1 Method Filename

This is the full path name of the file corresponding to the Method shown.

If the Method has been loaded from a file, this is the path name of the *source* file.

If the Method has been saved on a file, this is the path name of the *destination* file. The two files normally coincide. This field cannot be edited directly in the table (the field is read-only); the name of the Method file is assigned through the File Selection Dialog Box associated to the command `Method Open` and `Method Save As`.

7.2.2 Integration Parameters

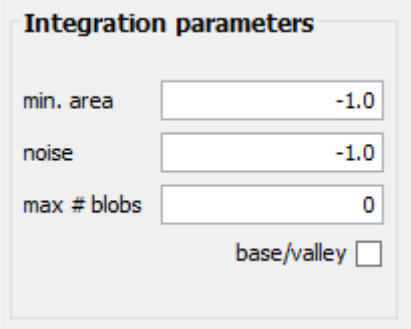


Figure 7.2: The Method Panel - Integration Parameters

This section contains three parameters and a check box; the parameters have initially values -1.0, meaning that the integration algorithm will assume default values. The parameters are:

- **Minimum Area**: minimum value of the area of the integrated Blobs (Blobs with smaller area will be ignored).
- **Noise**: value for filtering the noise level.
- **Max. number of Blobs**: it is used to limit the size of the Blob Table; Blobs will be sorted for descending Area values, and only those that fall in this number will be taken into account.

The check box **base-valley** allows to select the behavior of the integration algorithm calculating the base line in case of adjacent or coeluited peaks: if the box is checked, *base-valley* baseline is taken; otherwise *valley-valley* is used. The default

is unchecked, i.e. valley-valley, in order to maintain the compatibility with former versions of the software,

The figures below show the two types of integration

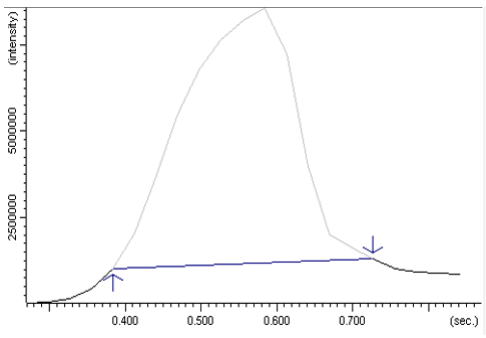


Figure 7.3: The Method Panel - Integration Parameters - Valley-Valley

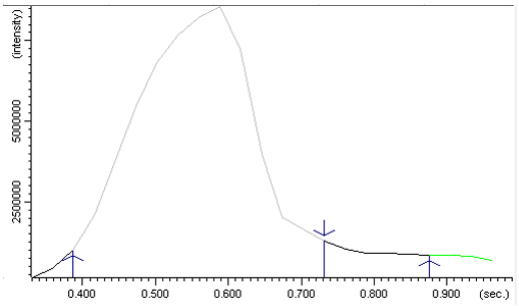


Figure 7.4: The Method Panel - Integration Parameters - Base-Valley

7.2.3 Spectra Parameters for Blob recognition

This section contains two sets of parameters.

- The left set is about the *Similarity*. It contains:
  - Check box and Text field **Minimum Similarity**. When the box is checked, the user should also specify a numerical value, expressed as a *percentage*. If spectra information is available, this is the minimal similarity that a peak must have to the library spectrum to be recognized.
  - Radio buttons **TtR Time Tolerance**, **2tR Tolerance** and **LRI Tolerance**; the user can choose between three types of tolerance criteria for Blob recognition:

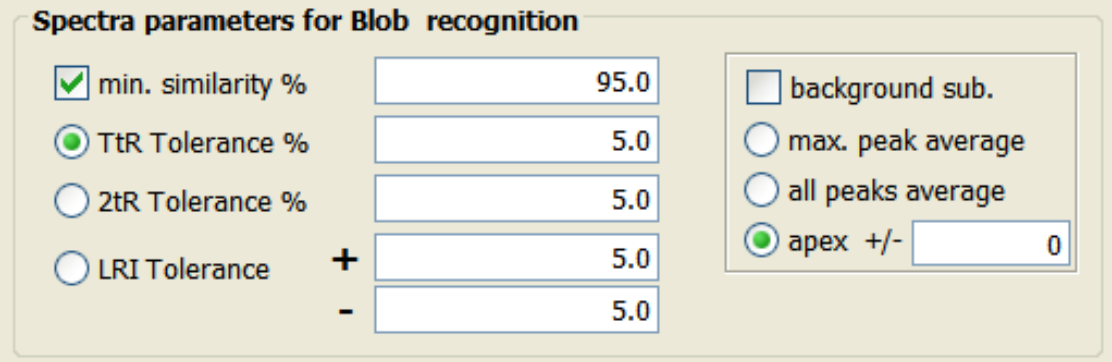


Figure 7.5: The Method Panel - Spectra Parameters

  * When **TtR (Total Retention Time) Tolerance** is selected, a corresponding percentage tolerance value should be specified in the text field at the right; only one tolerance value is requested, which will be used both for *upper* tolerance and *lower* tolerance. The value is the tolerance of the total retention time associated to the spectrum similarity to validate the recognition against the library.
  * When **2tR (Second Retention Time) Tolerance** is selected, a corresponding percentage tolerance value should be specified in the text field at the right; only one tolerance value is requested, which will be used both for *upper* tolerance and *lower* tolerance. The value is the tolerance of the second retention time associated to the spectrum similarity to validate the recognition against the library.
  * When **LRI Tolerance** is selected, two corresponding percentage tolerance values should be specified in the text fields at the right; in this case it is possible to distinguish the upper from the lower tolerance, by assigning different values. The values are the upper and lower tolerances of the linear retention index associated to the spectrum similarity to validate the recognition against the library.

- The right set describes the *Blob Spectrum* calculation; it contains:
  - Check box **Background Subtraction**; this control is used to activate and deactivate the subtraction of the *background* spectrum from the Blob spectrum before the library search.
  - **Radio buttons** which allow to select among different types of calculation of the Blob Spectrum; the resulting average spectrum may be background subtracted or not according to the above check box. The following types are provided:

Maximum Peak Average: the Blob Spectrum is calculated as the average of the spectra corresponding to the largest peak of each Blob.

All Peaks Average: the Blob Spectrum is calculated as the average of the spectra of all peaks of each Blob.

Apex: the Blob Spectrum is calculated as the average of the spectra of the scan points corresponding to the *apex* of each Blob; if a numerical value is also specified, the spectrum is calculated as the average of the spectra of a range of scan points around the apex of each Blob.

7.2.4 Blob Assembly Parameters

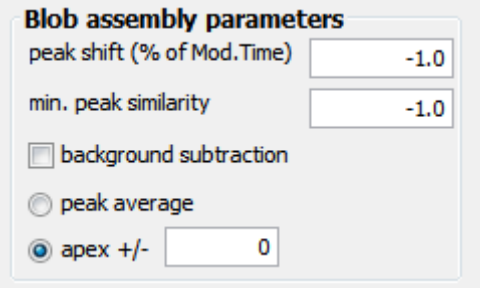


Figure 7.6: The Method Panel - Blob Assembly Parameters

This section contains the parameters used for the fine tuning of the Assembly of the Blobs. They are:

- **Peak Shift**: the maximum shift between two apexes of peaks to be assembled in the same Blob. The value is expressed as a *percentage of Modulation Time*.
- **Minimum Peak Similarity**: is the minimum similarity of the reference spectra of two peaks to be assembled in the same Blob.
- Check-box **Background Subtraction**: when checked, the *background* spectrum will be subtracted before assembly. The background is calculated according to the selection of the radio buttons below.
- Radio Button **Peak average**: background spectrum is calculated as the average of the spectra of all the scans in the peak.

- Radio Button **Apex +/-**: background spectrum is the spectrum of the apex; if a number of scans is also specified in the adjacent text field, the background spectrum is taken as the average of the spectra of a range of scan points around the apex of the peak.

7.2.5 Blobs ID Panel

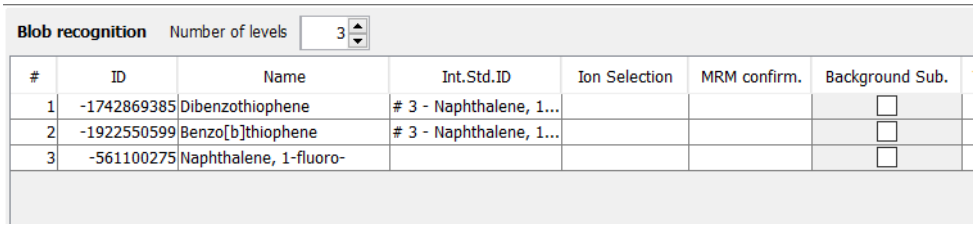
This Panel include the Table, some buttons for the table management, and a combo box used to assign the number of levels.

7.2.5.1 Blobs ID Table (Blob Recognition)

The `Blobs ID Table` contains a list of the Blobs IDs that will be used by the software for the Blobs recognition.

Starting from Release 2.0, *Chrom*^{square}stores all information about the concentrations of the standards for the Calibration inside the Method Panel. For this reason, the Blobs ID Table must provide a single column for each concentration level.

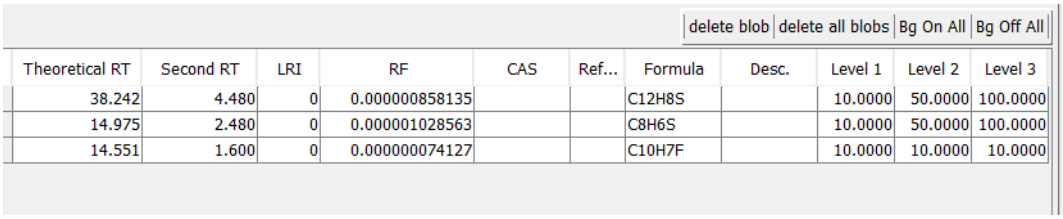
The Table is shown in the 7.7 and 7.8 (the figure is split in two parts for a better readability).



Blob recognition Number of levels 3

#	ID	Name	Int.Std.ID	Ion Selection	MRM confirm.	Background Sub.
1	-1742869385	Dibenzothiophene	# 3 - Naphthalene, 1...			☐
2	-1922550599	Benzo[b]thiophene	# 3 - Naphthalene, 1...			☐
3	-561100275	Naphthalene, 1-fluoro-				☐

Figure 7.7: The Method Panel - Blobs ID Table (left side)



delete blob | delete all blobs | Bg On All | Bg Off All

Theoretical RT	Second RT	LRI	RF	CAS	Ref...	Formula	Desc.	Level 1	Level 2	Level 3
38.242	4.480	0	0.000000858135			C12H8S		10.0000	50.0000	100.0000
14.975	2.480	0	0.000001028563			C8H6S		10.0000	50.0000	100.0000
14.551	1.600	0	0.000000074127			C10H7F		10.0000	10.0000	10.0000

Figure 7.8: The Method Panel - Blobs ID Table (right side)

The Table contains:

- a row for each Blob ID;
- **11 fixed columns**: `#`, `ID`, `Name`, `Int.Std.ID`, `Ion Selection`, `MRM confirmation`, `Background Subtraction`, `Theoretical RT`, `Second RT`, `LRI`, `RF`.

  1. The first column (`#`) is just a row counter; it is used only for reference purposes. This column is read-only.
  2. The next column (`ID`) contains the ID of the Blobs; normally, the ID is generated automatically. However the value may be changed by the user.
  3. The next column (`Name`) contains the names of the Blobs; the names are normally assigned by the user in the Blob Definition procedure (see 6.2.4.2). Blob names can be automatically generated using the `Search Selected Blob` or `Search all Blobs` commands. The table allows the user to modify the Blob names after their original definition.
  4. The next column (`Int.Std.ID`) is only used for Calibrations with Internal Standards. For each row, the cell corresponding to the third column indicates another Blob ID (among those defined in the Method) that will be assumed as internal standard. In order to simplify this assignment, the cells of the third columns are in effect *combo boxes*: each combo box reports a list of all other Blob IDs; the combo box allows the user to establish the mapping between the Blob ID and the Blob ID used as Internal Standard. Internal Standard IDs can be different for each row. This column is always user-defined.
  5. The next column (`Ion Selection`) is used to specify Ion Selection in the case of chromatograms generated by mass spectrometers; see Chapter 11.7 for details.
  6. The next column (`MRM confirmation`) is used to specify a MRM filter in the case of chromatograms generated by mass spectrometers; see Chapter 11.7 for details.
  7. The next column (`Background Subtraction`) is used to switch On/Off the subtraction of the background of the Blob ID. When selected, the background is measured before each peak start and after each peak end. The average spectrum of the background is subtracted when the total area of the Blob is calculated. This selection has no effect if a *Ion Selection* is defined for the given Blob. When background subtraction is selected, the baseline calculated upon the TIC is substituted by the average background total count. This column is always user-defined.

8. The next column (`Theoretical RT`) is used to show the Theoretical Retention Time of the Blob ID. This column is automatically filled by the software, but may be changed by the user.
9. The next column (`Second RT`) is used to show the Second Retention Time of the Blob ID. This column is automatically filled by the software, but may be changed by the user.
10. The next column (`LRI`) is used to show the Linear Retention Index of the Blob ID. This column is automatically filled by the software, but may be changed by the user.
11. The next column (`RF`) is used to show the Response Factor of the Blob ID. This a result of the Calibration procedure. This column is automatically filled by the software, but may be changed by the user.

- **4 optional columns**, containing the following information about the compound:

  1. CAS.
  2. Internal Reference Code.
  3. Formula.
  4. Description.

  In the case where the Blob ID has been identified through comparison with an external spectra library containing one or more of this information, they are retrieved from the library and shown in the table; in all other cases, the information can be supplied by the user. A configuration option allows to enable or disable the visualization of these columns.

- A variable number of columns, each one representing a *Concentration Level*. A Concentration Level describes a *Standard*, by assigning a set of concentration values, one for each Blob ID.

  The Number of Levels is assigned through a spinner control (7.2.5.2). The Number od Levels can be zero: in this case no variable column is present in the table, and no Quantitative Analysis is possible.

  The variable columns are typically filled by the user, which describes in this way the features of the Calibration. See 9.

When the user defines a new Method, rows are generated dynamically: as soon as the user defines a new Blob, a corresponding new row is added to the table, using the name assigned in the Blob Definition procedure (see 6.2.4.2).

See Sections 3.1.5, 3.1.6, 3.1.7 for more details about Blobs and Blob IDs.

7.2.5.2 Selector of the Number of Levels

This is a *spinner* control (that is a text field with two small arrows that allows the user to quickly assign and modify a integer number representing the *Number of levels* to be used in the Calibration.[1]

The Number of Levels corresponds to the variable columns representing the concentrations, placed at the right side of the Table.

The spinner control is synchronized with the columns: at each increment of the Number of Levels a new column is added after the existing ones, whereas at each decrement the rightmost column is deleted.

7.2.5.3 Buttons for Blob ID Table management

These buttons are placed near the upper right corner of the table. They are used for table editing:

- Button `delete blob`: it is used to delete the currently selected Blob ID (row of the table); take note there is no corresponding *add* button, since Blob Ids (rows) may be added to the table only through the aforementioned procedure (see 9.2.3).
- Button `delete all blobs`: this commands is used to delete all table rows.
- Button `Bg On All` (Background On All): pressing this button, all Background Subtraction check boxes are set to `On`, that is the background subtraction process is scheduled for all Blob IDs.
- Button `Bg Off All` (Background Off All): pressing this button, all Background Subtraction check boxes are set to `Off`, that is no background subtraction process is scheduled.

7.2.5.4 Blob ID Table Operations

Some facilities have been provided, in order to help the user to manage the Table, especially in the case of large ones (high number of Blob IDs and/or levels).

[1] Note that the *Number of Levels* does not generally correspond to the *Number of Standards*, since the same Level may be used for more Standards.

- **Assigning the same value to more cells**. This facility applies only to the columns representing the concentration levels. To enter the same value in more cells:

  1. Left-Click the mouse in the first (upper) cells.
  2. Write the value, followed by the *Enter* key.
  3. Right-Click the mouse in the same cell.
  4. From the context menu that will appear, select `Fill Down`. The cells placed in the lower rows of the same column will be filled with the same value. Filling will stop at the first not-empty cell.

  Due to the rule of stopping at the first not empty cell, in the case the user wants to define blocks of cells having the same value, the best thing is to start from the lowest cells and continue towards the top of the column.

  For example:

  - Let us consider a case with 10 rows, that we want to fill in the following way: 10, 10, 10, 20, 20, 20, 30, 30, 30, 30.
  - Go to row number 7, enter the value 30, and then give the command Fill Down; the rows 8, 9 and 10 will be filled with 30.
  - Go to row number 4, enter the value 20, and then give the command Fill Down; the rows 5 and 6 will be filled with 20; filling will stop at row 7 since a value is already present.
  - Go to row number 1, enter the value 10, and then give the command Fill Down; the rows 2 and 3 will be filled with 10; filling will stop at row 4 since a value is already present.

- **Clear Column**. This facility applies only to the columns representing the concentration levels. It allows to quickly delete all values of a column (it is normally used to prepare a clear set of cells for a subsequent use of the *Fill Down* facility).

- **Automatic Resize of columns**. Due to the large number of the columns of the Table, a good visualization may be sometimes problematic, especially in the case of many levels. The following facility has been set up, in order to allow the user to easily switch between two kinds of column sizing:

  - Initially, the *sizes of the columns have default values*, which allow a good visualization of all cell contents; in many cases (it depends on the resolution of the screen and on the size chosen for the Method Panel)

this does not allow the visualization of all columns and it is therefore necessary to use the *horizontal scroll bar to move right and left* the block of columns.

- Double-click with the left mouse button on the header zone of the Table will produce the effect of resizing the columns in such a way that *all columns are visualized at the same time*; as side effect, the contents of some cells could result shown in a shortened way; on the other hand there is the advantage of seeing the whole table at a glance.
- Double-clicking again, the visualization will return to the initial one.

7.2.6 Groups Panel

7.2.6.1 Groups ID Table

Groups ID Table contains a set of Group IDs that will be used by the software for the recognition of the Group IDs. The Table contains:

- a row for each Group ID;
- three columns: ID, name, Blob ID.
  1. `ID`: a counter of the Group ID.
  2. `name`: the name assigned by the user to the Group ID.
  3. `Blobs`: the list of the Blob IDs which belong to the Group ID, comma separated.

Note that the table is read-only, and all modifications should be accomplished through the buttons.

7.2.6.2 Button for Group Table management

Below the Group Table there are 2 buttons, to be used for table editing:

- Button `add`: is used to add a new row (that is a new Group ID) to the table; pressing this button will open a window containing a list of all currently defined Blob IDs; the user can then select through the mouse one or more Blob IDs that will become part of the Group ID. Pressing the CTRL key during the selection will allow to select more than one Blob ID.
- Button `delete`: it is used to delete from the table the Group ID currently selected .

7.2.7 Lower Panel

7.2.7.1 ROI

ROI means *Region Of Interest*; this defines a smaller region of *rectangular* shape inside the chromatogram Map.

The ROI is defined by the user through mouse commands and Context menu in the Chromatogram Panel (see 6.2.4.3).

The ROI is unambiguously defined by the *Total Retention Times* of two *opposite points* of the rectangle (remember that Total Retention Times actually identifies a *point* in the 2D Map Plot).

7.2.7.2 Ion Selection for Map Display

In this field the user can specify a list of Ions which will be used for the graphical representation on the chromatogram Map. Ions are represented with the same syntax used to select ions for quantitation (see Chapter 11.7).

Once this parameter has been set, it is possible to display the Chromatogram map with a color representation given only by the selected ions.

7.3 Shimadzu Spectra Search page

The window for the `Shimadzu Spectra Search` is shown in the Figure 7.9:

In order to perform the Shimadzu spectra search, GCMSsolution, LCMSsolution or LabSolutions must be installed. Please read the *Prerequisites* (Section 2.1) in order to correctly set up the Shimadzu software.

7.3.1 Library file #n

The page contains five edit fields,where the user can specify the full paths of the spectra libraries that the Shimadzu software will use to perform the spectra search.

The assignment of the paths is accomplished by pushing the little buttons representing a folder placed on the right of any text field. The buttons cause the opening a File Selection Dialog Box, which allows the user to select the library files (.lib) navigating across the Shimadzu software folders.

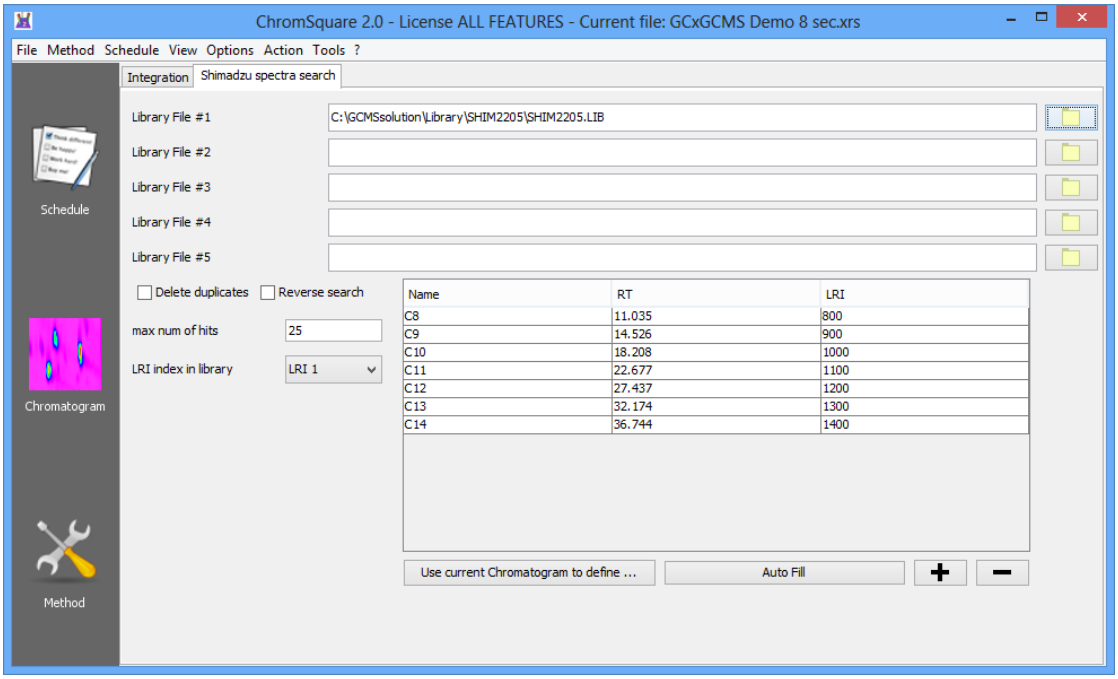


Figure 7.9: The Method Panel - Shimadzu Spectra Search Page

Shimadzu software allows to perform the search using up to 5 libraries. At least one library must be defined.

7.3.2 Delete duplicates

If this box is checked, the duplicate hits found during the search are deleted and only the one with the higher similarity index is displayed.

7.3.3 Reverse search

If this box is checked, the *reverse search* is performed instead of the direct one.

7.3.4 Max. number of Hits

This field is used to set the maximum number of compounds that will be returned by the Shimadzu software search. Default is 25.

7.3.5 LRI index in library

Shimadzu libraries allow to store up to 5 Linear Retention Indexes for each reference compound. This parameter lets the user to choose which one has to be used for the library search. If `no LRI` is selected, no filtering is performed against the Linear Retention Index.

7.3.6 LRI Table

This table is made by three columns:

1. **Name** is the name of the Blob identifying the LRI milestone (e.g., the appropriate alkane). This is only for a documentation purpose (and to remind the user): in fact, it is equivalent to write C7, Carbon 7, Heptane etc.
2. **RT** is the retention time of the top of the milestone Blob.
3. **LRI** is the retention index (conventionally set to the number of carbon atoms multiplied by 100).

The table is fully editable, so that the user can enter a new line (using the button "+" on the bottom of the table), delete a line (using button "-") and fill in the values. Once the table is filled with all values, when an integration is performed, the retention times for the Blob identified in the chromatogram are rescaled to the times included in the LRI table, and the LRI is computed for each Blob.

The user can also prepare a chromatogram containing the milestone Blobs (for example, an appropriate mix of alkanes) and integrate it. Once this operation is done, having the datafile open, it is possible to import those Blobs in the table by pressing the button "Use current Chromatogram to define LRIs". The table is automatically filled in, and the user must only fill the LRI for each entry. At this point the table is anyway completely editable, so it is possible to change the names or make corrections.

The button `Auto Fill` may be used to simplify the data entry process: once the user has compiled a given row, subsequent rows may be automatically assigned by the software, until a not empty row is found.

Once the Method is saved, all Blobs which do not have an assigned LRI are deleted from the table, since they do not contribute to the computation.

For the Blobs that are outside the RT interval between the first and the last entry of the table, the LRI value is calculated by *extrapolation*.

7.4 Measurement page

This page is used to define the *precision* used in the representation of a set of parameters: this is defined in terms of *granularity*, that is the smallest amount of the parameter that will be taken into account.

A precision of 1.0 means an approximation to an integer value, a precision of 0.1 means an approximation to one decimal digits, and so on. Precision is however a more general concept than the number of decimal digits, since it can assume any value and not only the negative powers of 10 (0.1, 0.01, etc.).

The parameters and their precisions are arranged in a two-columns table: the first column(*Quantity*) is fixed and identifies the parameter, the second column (*Precision*)is used for entering the desired precision value.

Presently only one Measurement parameter is defined:

- **Precision-amu**: this is the precision of the m/z value in the description of Mass Spectra, expressed in *Atomic Mass Units*. This parameter is used for handling spectra with *Exact Mass* modality, that is spectra having Mass values represented with arbitrary precision.

A sample of Measurement page is shown in 7.10.

7.5 UV Settings page

This page is used for setting some parameters used in the *Similarity calculation of UV spectra*.

Presently the two following parameters are defined:

- **Start wavelength** (nanometers): the initial point of the wavelength range used for similarity calculation in UV spectra.
- **End wavelength** (nanometers): the final point of the wavelength range used for similarity calculation in UV spectra.

If the two values are zero, no wavelength filtering is done, and the similarity calculation is performed on the whole wavelength range. Modifying the limits, that is assuming a narrower range of wavelengths, will emphasize the differences in similarity indexes.

A sample of UV Settings page is shown in 7.11.

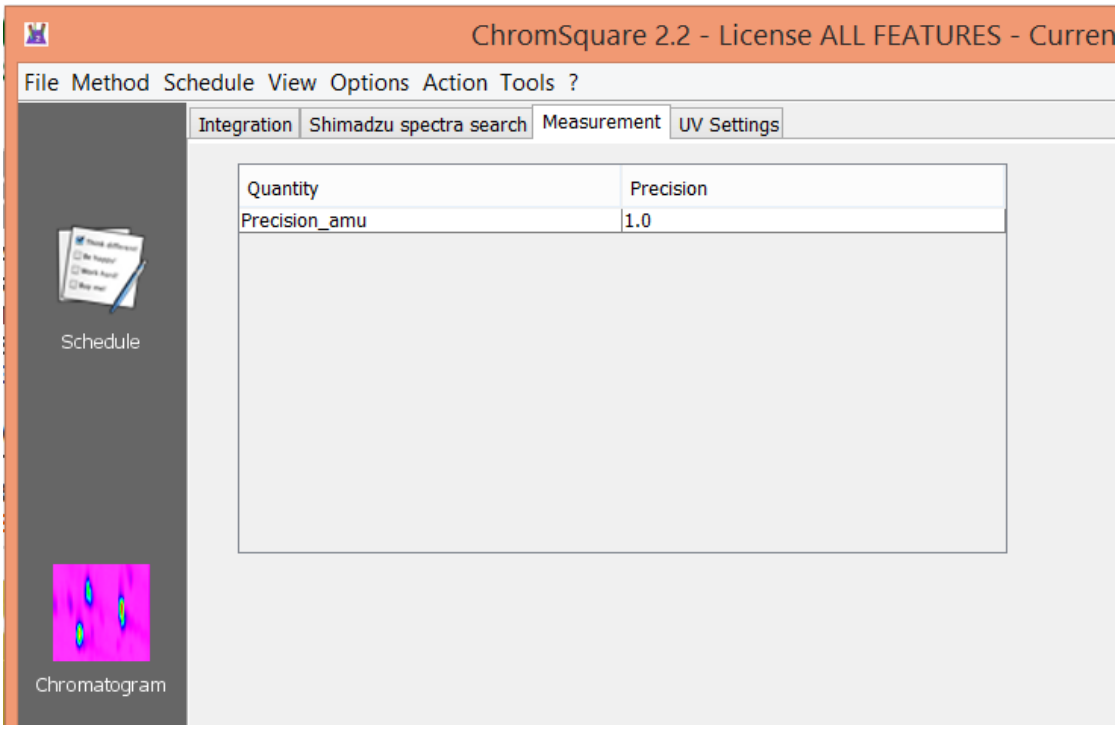


Figure 7.10: The Method Panel - Method Measurement Page

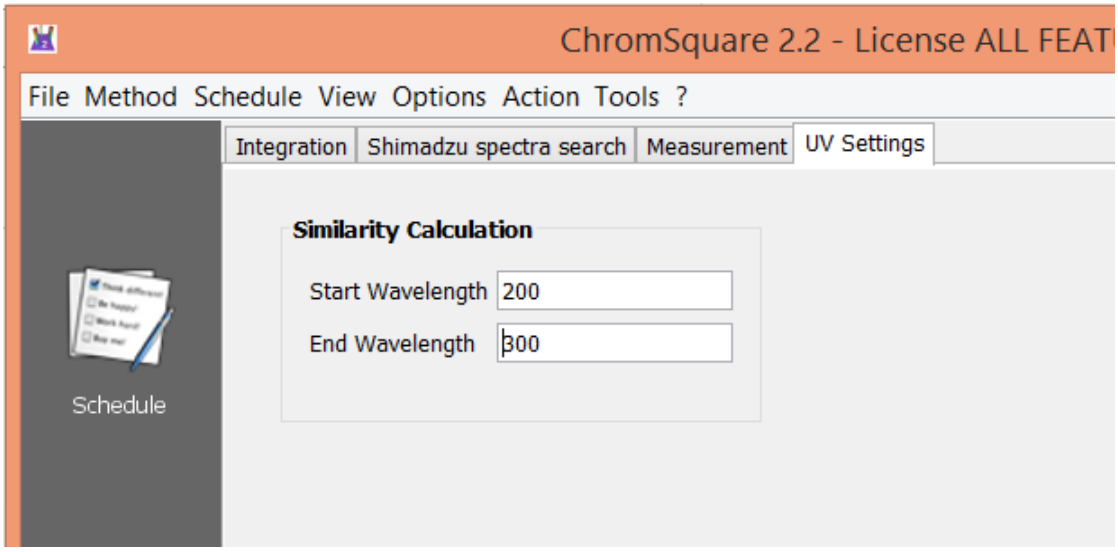


Figure 7.11: The Method Panel - UV Settings Page

Chapter 8

Qualitative Data Analysis

In this chapter it will be presented a summary of the operations of a routine *Qualitative Analysis*. In order to maintain the description fluent, only the key points will be here reported, with frequent references to specific sections of the manuals

8.1 Set up the Chromatogram

8.1.1 Load the Chromatogram

Use the menu command `File | Open Datafile` (see Section 4.1.1) to load a chromatogram into memory.

For large chromatograms the loading may require some time (several seconds or even minutes); a *progress bar* is always displayed, giving a rough estimate of the time required to complete the process.

When the loading operation has finished, the progress bar window closes and the chromatogram is automatically displayed in the Chromatogram Panel.

8.1.2 View the Chromatogram

After a little expertise, the user is generally able to view at a first sight whether the displayed Map Plot is correct or not. Some "anomalous" patterns are easily recognized and may suggest corrections to some parameters from the user.

Modulation Time and *Wrap Around* are the two parameters to be influenced.

8.1.2.1 Correct Modulation Time

A wrong modulation time is generally the main responsible of an anomalous Map. This is the most common case, since this information cannot be supplied automatically.

The user can easily change it and reload the chromatogram to view the changes in the Map Plot. See Section 4.1.1.3.

Note: after the first loading of a Data File, the software generates the corresponding Result File (see 3.3.2). If the user tries to load again the Data File using a different Modulation Time, the software warns the user that the Result File is already present and asks whether he wants to overwrite it or not. In this case the answer should be "Yes".

8.1.2.2 Correct Wrap Around

The *Wrap Around* correction does not change the Blob patterns, but moves the whole Map Plot towards the top or the bottom.

See Section 4.6.15.

8.2 Integrate the Chromatogram

The integration of a new chromatogram includes various steps:

1. set up the Method;
2. integrate the whole chromatogram;
3. (optionally) define a smaller area (Region of Interest, or ROI) and integrate only this one;
4. check the integration results;
5. (optionally) modify some integration parameters.

For new types of chromatograms, the various steps may be repeated more times. Once satisfactory results are obtained, the Method can be saved and used for the integration of similar chromatograms (this is normally the case in Quantitative Analysis). The integration with an established Method is a straightforward activity which involves just one click.

8.2.1 Set up the Method

Setting up the Method includes the following actions:

1. Switch the main view to the Method Panel, using the `Method` command in the Side Tool Bar. Method Panel is thoroughly described in Chapter 7.
2. Use an existing Method, loading it by a Method File, either create a new Method from scratch:
   - To open an existing Method, use the menu command `Method|Open` (Section 4.2.2) and select a "`.xcm`" Method file through the File Selection Dialog Box.
   - To create a new Method, use the menu command `Method|New` (Section 4.2.1).
3. Define the three Method parameters (shown in the Figure 8.1):
   - **Minimum Area**: the minimum value of the area of the Blobs that will be integrated (Blobs with smaller values will be discarded).
   - **Noise**: value of the noise level for Blobs filtering.
   - **Max. number of Blobs**: maximum number of Blobs that will be shown on the Results table (Blobs will be initially sorted by descending area values: only the larger ones will be taken into account);

   In the case of a new Method, the first two fields are initially filled with the value -1.0, which means that a default value is assumed for each parameter; the third field is set to 0, meaning that no Blob number filtering will be done.

   In the case of an existing Method, the values assigned in a previous work session are taken.

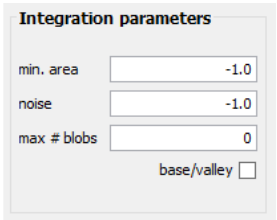


Figure 8.1: The Method default integration parameters

8.2.2 Integrate Whole Chromatogram

To integrate the whole chromatogram:

- either use the menu command `Action | Integrate Whole Chromatogram` (Section 4.6.2);
- either use the *first* icon of the Data Analysis Tool Bar (Section 6.5.3)

Figure 8.2 shows a samples of whole chromatogram integration.

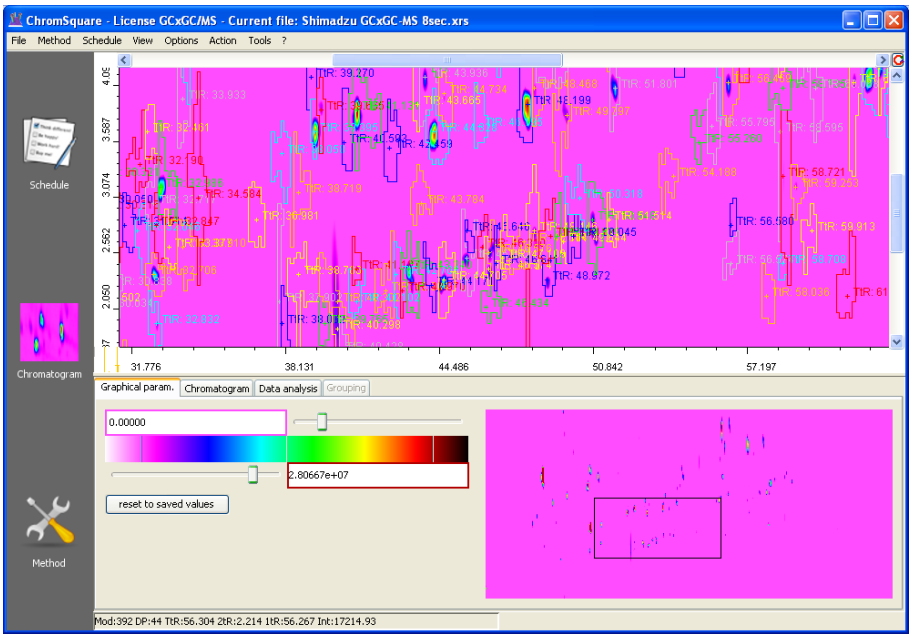


Figure 8.2: Qualitative Analysis: Whole Chromatogram integrated

8.2.3 Integrate ROI

To integrate a ROI:

- Define the ROI, if this has not already been defined (the *ROI* is stored in the Method, and its definition can be viewed in the Method Panel, as the Total Retention Time values of two opposite points; see 7.2.7.1). To define a ROI, use the Map View Selection Context Menu (see Section 6.2.4).
- Perform the ROI integration:

- either use the menu command `Action | Integrate ROI` (Section 4.6.1);
- either use the *second* icon of the Data Analysis Tool Bar (Section 6.5.3)

Figures 8.3 and 8.4 shows samples of a ROI definition and its corresponding integration.

8.2.4 Check the Integration Results

After the integration (both in the whole or the ROI cases) the user may check whether the results are correct (in the routine analysis this step is not necessary, but in a new analysis it may be important).

To check the analysis result the user may:

- View the integration results - that is the representation of the integrated Blobs - on the 2D Map Plot; in order to improve this visualization, it is then possible:
  - to *zoom* on particular zones of the plot (see Section 6.2.3);
  - to change the *details* of the Blob representation, through the various items of the `Menu Options` (see Section 4.5);
  - to change the *Color Map*, as described in the Section 6.3.1.

  All operations described above are managed in real time (that is the map is automatically updated and re-drawn without performing any additional operation: no further integration is required).
- View the integration results as *numerical values* in the Data Analysis window, which is fully described in Section 6.5.
- View the *linear chromatogram profile* in correspondence of the single Blobs, as described in Section 6.4.

8.2.5 Change the Integration Parameters

If the results checking described above is not satisfactory, the user can change some integration parameter and repeat the steps described in Sections 8.2.1, 8.2.2, 8.2.3, 8.2.4.

The integration parameters that can be changed are the Method parameters, described at 8.2.1.

Figure 8.3: Qualitative Analysis: Define ROI

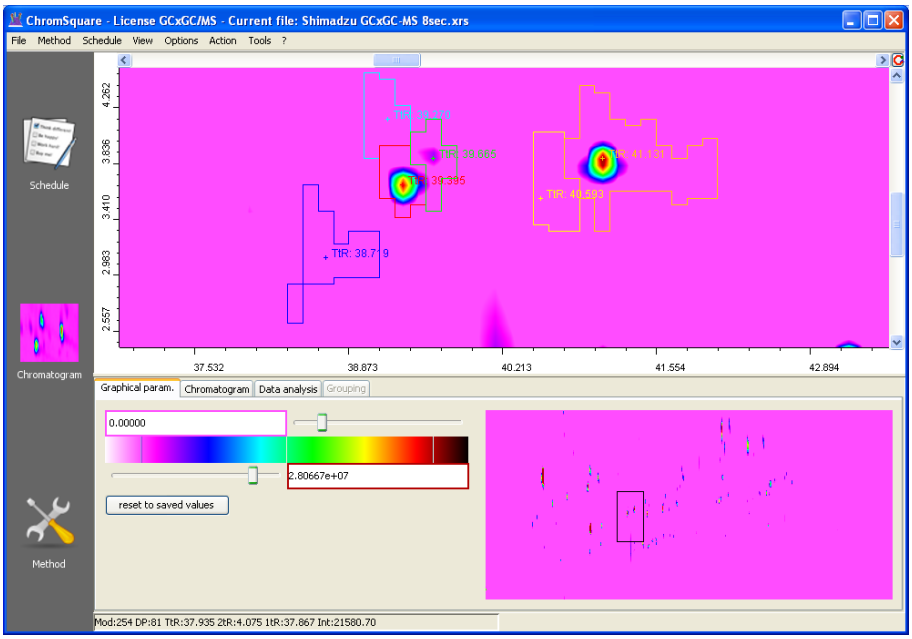


Figure 8.4: Qualitative Analysis: ROI integrated

8.3 Identify the Blobs

The identification of the Blobs (that is, the operation of assigning a Blob Id to the Blobs) can be performed in two ways:

- Manual identification.
- Automatic identification.

8.3.1 Manual Identification of the Blobs

Manual identification is obviously the only identification possible when the current software license does not provide the link with the software which performs the identification (see next section for details about this).

Manual identification may be also recommended when the user is interested to few well known and defined Blobs, which are recognized at a first sight, and prefers to avoid the automatic process, that in some case may be time-consuming.

Manual identification must be made separately for each Blob to be identified.

The Manual identification is made up by two steps:

- In the first step, the user defines the region which includes the Blob; this may be a *rectangular* region or an arbitrary *polygonal* region.
- In the second step, a dialog box allows the user to write the name to be assigned to the Blob.

The procedure will be reported in details for the two cases.

8.3.1.1 Rectangular Regions

To perform the Manual identification of Blobs included in a *rectangular region*, follow these steps:

- Define the Blob region, according to the instructions reported in Section 6.2.2 and 6.2.4.
- Enter the Blob name in the dialog box that will be displayed soon after, as described in Section 6.2.4.2.

If the selected region contains more than one Blob, the same name will be assigned to all them.

8.3.1.2 Polygonal Regions

To perform the Manual identification of Blobs included in a *polygonal region*, follow the procedure described in Section 11.4.

If the selected region contains more than one Blob, the same name will be assigned to all them.

8.3.2 Automatic Identification of the Blobs

The automatic identification of the Blobs can be performed in the cases in which the current license provides a link with an external software which can execute the task.

Presently the following licenses provide this feature:

- GCxGC/MS & LCxGC/MS version for Shimadzu GCMSsolution and GC-solution (using Shimadzu Search engine).
- LCxLC version for Shimadzu LCMSsolution and LabSolutions LCMS (using Shimadzu Search engine).
- LCxLC version for Shimadzu LabSolutions LCMS (using Shimadzu Search engine).
- Text and NetCDF Version with NIST Spectra Search (using NIST Spectra Search engine).
- All Features Version (using Shimadzu Search engine or NIST Spectra Search engine).

The present license provides the Automatic Identification of the Blobs.

The automatic search includes from one to three steps:

1. the first step consists of the command which starts the search;
2. the second step is optional and allows the user to modify the identified Blob;
3. the third step is also optional, and allow the user to add one or more identified Blobs to the Method.

8.3.2.1 Search the Blobs

The automatic Blobs search can be performed in two modes:

- searching *a single Blob at a time*;
- searching *all Blobs* with one command.

The first one is preferred in new cases, when the user wants to check libraries, computing times and so on. The second one is the preferred one in the daily analysis routine.

To search *one* Blob:

- Select the row corresponding to the Blob in the Data Analysis table;
- either give the command `Search Blob` of the Data Analysis Context Menu (see Section 6.5.4.1);
- or the command `Search Selected Blob` (Section 4.6.13) of the `Action` main menu.

To search *all* Blobs:

- give the command `Search All Blobs` (Section 4.6.14) of the `Action` main menu.

8.3.2.2 Select the Compound

This step may be executed when the user wants to modify one or more automatic Blob assignments, selecting a different compound from the list generated by the automatic search procedure.

The step is *optional* (in many case, the suggested compound is a good choice).

See Section 6.5.4.6.

8.3.2.3 Add Blob/s to Method

This step may be executed when the user wants to insert one or more Blob identifications in the Method.

The step is *mandatory* only if the current analysis will be followed by Quantitative Analysis.

See Section 6.5.4.8.

Chapter 9

Quantitative Data Analysis

In this chapter it will be presented a summary of the operations of a routine *Quantitative Analysis*. In order to maintain the description fluent, only the key points will be here reported, with frequent references to specific Sections and Figures of the manuals for further details.

The Chapter contains four Sections:

1. External Standard Calibration.
2. Internal Standard Calibration.
3. Quantitation of an Unknown Sample Chromatogram.
4. Group Calibration.

9.1 External Standard Calibration

9.1.1 Set up the Schedule

Chrom^{square} software can be used to build *Calibration Plots* by processing data analyses performed at different concentration levels, using pre-measured GCsolution or GCMSsolution files acquired in "`.gcd`" or "`.qgd`" data formats.

All files that enter in a Quantitative Analysis must belong to the same folder.

9.1.1.1 Rename the files

This Section reports some hints about the file names of the chromatograms, in order to simplify the operations and avoid errors[1].

Some file renaming could be then necessary, according to the following rules:

- The software orders the files alphabetically according to their names. The names should therefore reflect the logical order of the standards (concentrations and replication numbers).
- Avoid spaces in filenames; use underscore signs (_) or minus signs (-) instead. It is difficult to distinguish one space from more spaces if the characters are small; besides, spaces alter the alphabetic order.
- When using numbers to identify concentrations, try to always maintain the same number of digits. As an example, if you use 025, 050, 100, the standards will be always ordered correctly; if you omit the leading zeroes, the order may be wrong.

9.1.1.2 Define a New Schedule

Once your file list has been created, open *Chrom*^{square} software using the appropriate license and switch to the *Schedule Panel* using the schedule icon `Schedule` at the top of the left sidebar.

9.1.1.3 Add files to the Schedule

Use the `Plus Button` (that is is the icon with the *plus* sign at the left side of the Schedule Panel Tool bar) to add chromatograms to the current Schedule.

You must insert in the Schedule *all* chromatograms that should enter in the Calibration as *Standards*. You can also add one or more chromatograms that should be treated as *Unknown Samples*; the latter ones, however, may be added in a second time.

You must *add one Standard chromatogram at a time*, using an *increasing* order of concentrations. This is not mandatory, but it will help to write down the corresponding concentrations.

[1] The software actually accepts any kind of name for the files; anyway, if names are scrambled, it will be more difficult to place them in the right order.

Alternatively, you can add chromatograms to the Schedule by selecting the `Schedule|Add Chromatograms` command from the Menu Bar.

Files can be removed from the current Schedule using the Minus Button (this is the icon with the *minus* sign at the left side of the Schedule Panel Tool bar).

Note that *adding* a chromatogram to the Schedule does *not* mean *loading* it: adding just creates a reference to the file; the very loading will be accomplished later (see Section 9.1.3). Like removing a chromatogram from the Schedule does not mean to delete it, but just the link.

Use the *Arrows* to change the order of files in the current Schedule, i.e. to move upward or downward.

Other commands are available in the Schedule Menu, to *create* a new Schedule, *save* the current Schedule, or *open* an existing one (see Section 5 for a detailed description of the Schedule management).

9.1.2 Set up the Method

Once the Schedule has been completed, switch to the Method Panel, using the Method icon (at the bottom of the left sidebar).

If you already have a suitable Method for this calibration, load it through the menu item `Method|Open Method` (see Section 4.2.2). Continue to Section 9.1.2.2 if you want to modify some parameter; otherwise skip directly to Section 9.1.3.

If you do not have a Method, define it now though the menu command `Method|New Method` (see Section 4.2.1).

9.1.2.1 Define a New Method

After giving the menu command `Method|New Method`, the Method Panel should show an empty Method, that is most fields should be void (some fields will contain the value -1.0, which means a default value). If you see other numbers or words somewhere, a Method is already loaded; better to close all and start again.

9.1.2.2 Define Method Parameters

In the Method Panel, enter Minimum Area, Noise and Peak Shift.

9.1.3 Load a chromatogram

Once the Method has been set up, switch back to the *Schedule Panel*, select the first chromatogram and load it using the *icon with the Magnifying Lens* (third icon of the top tool bar). See Section 5.1.

Alternatively, you can load the chromatogram by *double clicking* on the selected row.

Loading chromatogram may require some time, during which a small window showing the progress of the operation is displayed.

At the end of the loading process the software will automatically switch to the *Chromatogram Panel*, where the loaded chromatogram will be shown.

See Chapter 6 for a full description of the Chromatogram Panel.

See also the various Sections of Chapter 8.1.2 for details about the operations that can be made to better view the displayed chromatogram.

9.1.4 Integrate the chromatogram

To integrate the chromatogram:

- either use the main menu command `Action|Integrate whole chromatogram`;
- or, in the lower window of the Chromatogram Panel, switch to the Data Analysis window and click on the first icon (the one with the "gear" symbol) of the Data Analysis Tool Bar (6.5.3).

Integration may require some time, especially in large chromatograms: a Calculation Progress window allows the user to monitor the status of the integration.

When the integration terminates, the Map Plot is redrawn, showing the boundaries of the Blobs and other information, which are displayed directly on the Map Plot at each Blob position.

At this point, before proceeding with the other steps, the user may use the various zoom functions to get a more detailed representation of some regions of the Plot, and also use the various items of the Menu Options (see Section 4.5) to change the details of the displayed information. Zooming also improves the readability of the information displayed on the Map (when too many Blobs have been recognized, the various info texts overlap; zooming has also the effect of "spreading" the Blobs, allowing to distinguish the single texts).

See also the Section 8.2.4 for various hints about the visualization.

9.1.5 Identify the Blobs

The identification of the integrated Blobs has been already described in details for Qualitative Analysis.

Two kinds of identification are provided: *manual* and *automatic*:

- Manual identification is always possible; it must be done separately for each Blob that should be included in the Calibration.
- Automatic identification is provided for some types of license, and it is performed on all integrated Blobs; in this case it is possible to use ROI (see 8.2.3) to limit the number of Blobs.

Skip to Section 8.3 for a full description of both procedures.

At the end of the identification, switch again to the *Method Panel*: you will find that the software has automatically filled the Blob Table in the Method, adding the names and the IDs of each identified Blob.

Once the identification has been completed, *save* the Method with the main menu command `Method|Save As`, choosing a file name and a folder for the Method. File name should have the extension "`.xcm`" (the software assigns it automatically at any case); folder may be any, but it is recommended to use the same folder which contains the calibration data files.

After the saving operation, the full path of the Method file will be shown in the Method Panel.

9.1.6 Integrate again

From the main menu, select the command `Action|Integrate Whole Chromatogram` again; at the end, the software switches to the Chromatogram Panel.

Now, since the Method contains a full definition of Blobs, the software can recognize all the Blobs of the chromatogram and show their names in the Map Plot.

Select the Data Analysis tag in the lower window to see the Data Analysis Table, which now contains also the names and the IDs of the Blobs which have been identified.

9.1.7 Integrate All Chromatograms

Analyzing the results of the second integration described in the previous Section, you had been able to verify whether the Method which has been previously set up and saved entirely fulfils the needs of the current calibration (in other words, if all Blobs necessary to the calibration have been correctly integrated and identified).

If this is the case, it is possible to proceed with the integration of all remaining chromatograms of the current Schedule, using the current Method. On the contrary, you should modify the Method, coming back to Section "Set Up the Method" (9.1.2) and repeat the various steps.

The integration of the remaining chromatograms may be done in two ways:

- *manually*, one chromatogram at a time,
- or *automatically* through the main menu command `Action|Integrate All Chromatograms` (see 4.6.5).

The first one may be preferred in "tricky cases", where may be useful to investigate every single chromatogram for possible anomalies. This way lets the user to check the results of every single chromatogram before continuing with the next one.

The second one is that preferred in the daily routine work. This is a powerful command that, for each chromatogram of the Schedule, performs the following operations:

- loads the chromatogram;
- integrates it;
- saves the results in the "`.xrs`" file.

All operation are carried on without further intervention of the user. The integration of many chromatograms can take a lot of time (obviously depending on number and complexity of the chromatograms), during which various images will flash in the graphical interface. Also various *monitor windows* will appear and disappear during the process.

The process cannot be interrupted by the user: at any case, once started, it should be left working and terminated by itself. Trying to close the monitor window has no effect on the whole process (this only closes the monitor window for that integration; a new window will be generated at the next integration step).

9.1.8 Assign the concentrations

Now you can return to the *Schedule Panel*. Nothing seems to have happened; this because when you added the chromatograms the Method was still undefined. In order to let the Schedule know that the Method has changed, it is necessary to give the menu command `Method|Open Method`, and reload the the Method that have been just saved.

You will see that new columns (one for each Blob) will be automatically placed in the table and temporarily filled with *zero values of concentration*.

Now you can write the concentrations in the cells of the Schedule table. **Rows are the Standards, columns are the Blobs**.

To facilitate the input, select a cell and give a double click: the cell will be shown in reverse and you can directly write on it. You can also navigate through the cells using the *Tab* key.

For each row of the Table, you must also specify whether it corresponds to a *Standard* or an *Unknown Sample*. Use the combo-box in the second columns to make this choice.

Note that rows corresponding to the *Standards* will be coloured in *green*; rows corresponding to the *Unknowns* will be coloured in *yellow*.

9.1.9 Save the Schedule

At this point you can save the Schedule through the menu command `Schedule|Save schedule as`.

Choose the same directory of the other files and a proper name for the Schedule. Schedule files have a "`.wls`" extension.

9.1.10 Create the Calibration

Now you have all the elements for making the Calibration:

- the concentration values of all Blobs of all Standards (assigned by the user at 9.1.8) are stored in the current Schedule;
- the area values of all Blobs have been saved in the "`.xrs`" files corresponding to each Standard (generated at 9.1.7).

Just select the menu item `Action|Create Calibration`.

The command performs the following operations:

- opens sequentially all the "`.xrs`" files and loads into memory the area data; this is a fast operation, since the "`.xrs`" files are relatively small files already containing all essential data;
- make the calculation of the Regression lines for each Blob, correlating the areas as linear functions of concentrations;
- shows a final summary of the calculation in a Dialog Box.

This summary is only a warning that the Calculation has been completed correctly (a diagnostic Dialog Box, showing the anomalies, will be generated otherwise). The full reporting of the results will be discussed in the next Section.

Press the Ok button to close the Dialog Box. If the Calibration terminated without errors, the window Calibration Data will open soon after.

9.1.11 View Calibration results

The Calibration Data window is made up by two pages (or tabs): *Calculation* and *Graphs*.

- The Graph page (a sample is shown in Figure 9.1) includes four *panels*:
  - The *upper-left* panel is a *List* of all Blobs included in the Calibration; the List is used to select a Blob at a time, whose characteristics will be shown in the other panels.
  - The *upper-right*panel is a *Plot* of the regression line corresponding to the currently selected Blob of the List.
  - The *central* panel is a *Table* which describes the characteristics of the input data used to build the regression of the currently selected Blob of the list; the Table contains a *row for each Standard* chromatogram; columns represent:
    1. The Blob name (this is equal for all Standards);
    2. the Id of the Standard (that is a counter);
    3. the Area of the Standard (this is the result of the Integration);

4. the Concentration of the Standard (this is the value assigned by the user in the Schedule Table; see the Section 9.1.8).

– The *lower* panel shows a *Report* of the calculations regarding the currently selected Blob of the List; the Report includes:

  * The coefficients of the regression line;
  * the Response Factor;
  * the Linear Correlation Coefficient (R) and R *squared*.

- The Calculation page contains a detailed report of the various calculations and results of the calibration in *numerical form*.

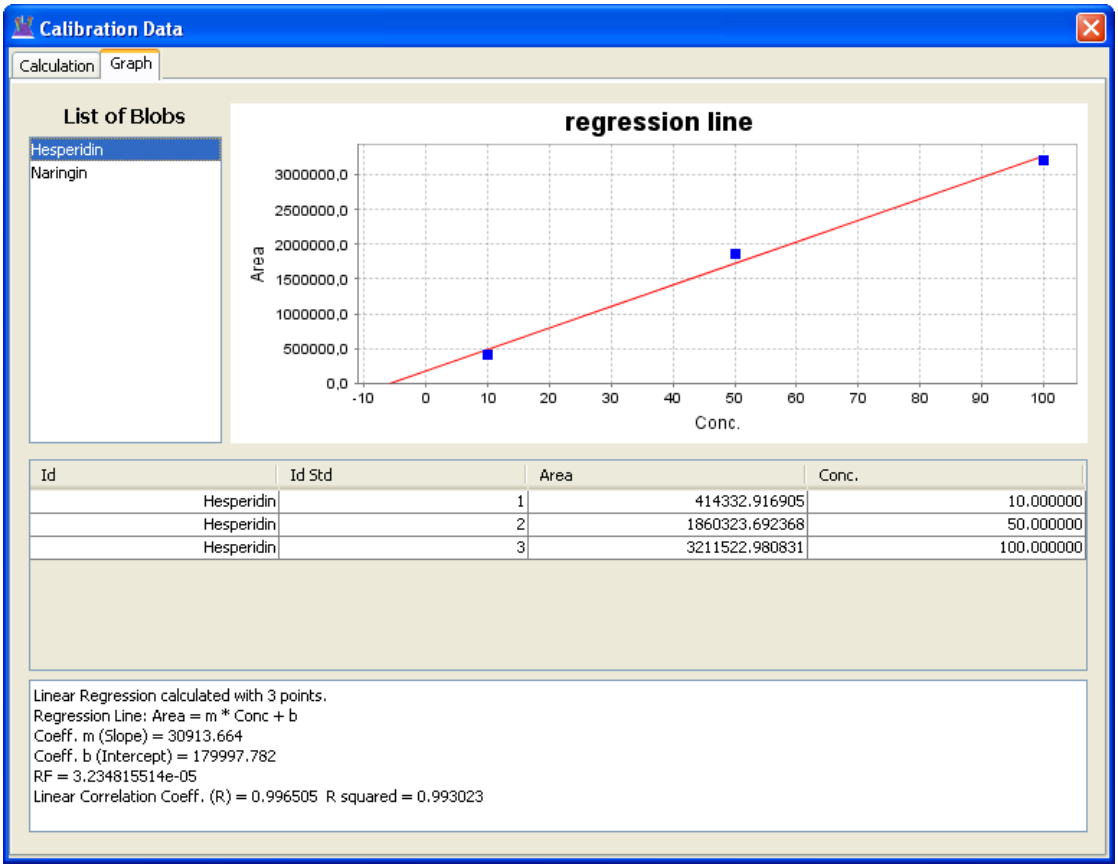


Figure 9.1: The Calibration Graph window

In the Graph Page, select the Blobs one at a time in the upper-left list, and check the *Regression Line* and the numerical coefficients in the Report Panel.

9.1.12 Save the Schedule again

Finally, save again the current Schedule through the menu command `Schedule|Save schedule as`, choosing the same file name used before (see Section 9.1.9).

This time, in the Schedule file will be also stored *the coefficients of all the Regression lines*. The Schedule file is now a *Calibration file*, that may be used for a Quantitative Analysis of an unknown sample.

9.2 Internal Standard Calibration

Calibration with Internal Standards is very similar to the one described for the External Standard in the Section 9.1.

In this Section, only the steps that differ from the External Standard will be discussed; for all other steps, it will only be reported a reference to the corresponding Section.

9.2.1 Set up the Schedule

See Section 9.1.1.

9.2.2 Set up the Method

See Section 9.1.1.

9.2.3 Load a chromatogram

See Section 9.1.3.

9.2.4 Integrate the chromatogram

See Section 9.1.4

9.2.5 Identify the Blobs

See also Section 9.1.5.

Once the Blobs have been recognized, they are enumerated in the Blob table of the Method Panel.

If a Blob has to be calculated using an Internal Standard, the cell corresponding to the `Int.Std.ID` column (the third of the table) must contain the *Blob ID of the other Blob that should be used as Internal Standard*. If the cells contains 0 or -1, this means that the Blob does not use the Internal Standard.

Clicking an any cell of the third column, a small Combo Box will be displayed, enumerating all available IDs. Select one of them (different from 0) to assign the Internal Standard. Select "0" to delete the Internal Standard assignment.

9.2.6 Integrate again

See Section 9.1.6

9.2.7 Integrate All Chromatograms

See Section 9.1.7.

9.2.8 Assign the concentrations

See also Section 9.1.8.

You will see that in the Schedule Table the columns relative to the *Blob defined as Internal Standards* are coloured in *green*, like the rows corresponding to the Standards.

You must fill in the *concentration of the Internal Standard in the sample chromatogram.*

Remember that green cells are those where the user can write the concentrations, whereas yellow cells are those where concentrations will be calculated.

9.2.9 Save the Schedule

See Section 9.1.9.

9.2.10 Create the Calibration

See also Section 9.1.10.

Note: in the regression line plot, the *concentrations and the areas are divided by the concentration and the area of the internal standard*, so they assume very low values (indicatively the magnitude of the unity).

9.2.11 View Calibration results

See Section 9.1.11.

9.2.12 Save the Schedule again

See Section 9.1.12.

9.3 Quantitation of an Unknown Sample Chromatogram

The Quantitative Analysis of an unknown sample (also called "Quantitation") is a very simple operation once that a Schedule file containing a Calibration has been set up.

9.3.1 Add unknown sample files to the schedule

Execute the following steps:

- Switch to the Schedule Panel.
- Add one or more sample chromatograms to the current Schedule, using the commands described at Section 9.1.1.3. If the sample chromatograms have already been added to the Schedule when defining the Standards, skip to the next Section.
- Define the chromatograms just added as "Samples", using the `Std.Smp.` combo box.

In the Schedule Table, rows corresponding to Samples have a *yellow* background (whereas Standards have a *green* background).

9.3.2 Load a chromatogram

The operation is the same described for Standards. See Section 9.1.3.

9.3.3 Integrate the chromatogram

The operation is the same described for Standards. See Section 9.1.4.

Since the current Method already contains the identification of the Blobs, the integration command should also recognize the Blobs.

This operations may be skipped if all unknown samples have been added to the Schedule together with the Standards: in this case, the command Integrate All Chromatograms (described at 9.1.7) will also integrate the unknown samples and save the corresponding "`.xrs`" files with the area data.

9.3.4 Execute the Quantitative Analysis

To execute the Quantitative Analysis of all Unknown Samples included in the Schedule give the main menu command `Action|Quantitate`.

The command will use the Calibration defined within the current Schedule. Error messages will appear if the Schedule does not contain a correct Calibration.

The Quantitation results will be shown in various places. Perform the following actions to see all results:

1. Switch to the *Schedule Panel*; the concentration results are shown, for each column corresponding to a Blob, on the yellow lines corresponding to the unknown Samples (these concentration were all zero before executing the Quantitation command).
2. From the Schedule Panel, *load* a Chromatogram corresponding to an unknown Sample (double click on the row or use the Magnifying Lens icon). The current panel automatically switches to the Chromatogram Panel.
3. Look at the Map Plot: the concentrations will be shown near to each Blob;
4. Look at the Data Analysis Table (6.5.1) in the Data Analysis window of the lower panel: the Concentration column will now be filled with the calculated concentration values.

9.4 Group Calibration

9.4.1 Description

The *Group Calibration* is a type of calibration based on groups of Blob IDs (a Blob ID, in its turn, is a group of adjacent Blobs, as described in Section 3.1.6); in order to distinguish the two kinds of groups, we will call the groups of Blob IDs as *Named Groups* or simply *Groups*.

Whereas a Blob ID is a set of one or more Blobs belonging to a given Region (rectangular or polygonal), a *Named Group is a set of more Blob IDs*, assembled by the user according to different criteria.

Both Blob IDs and Named Groups are defined inside the Method: whereas Blob IDs are defined by the user through a graphical procedure, Named Groups are defined at a second step, by manually assembling the Blob IDs defined in the former step.

The *Group Calibration* is similar to the normal Calibration (also called Blob Calibration), with the difference that it handles *Groups of Blob IDs* instead of single Blob IDs. This means that in the Calibration algorithm the area of a Blob ID is replaced by the sum of the areas of all Blob IDs included in the Group, and the concentration of a Blob ID is replaced by the sum of the concentrations of all Blob IDs included in the Group.

From the analytical point of view, the Named Groups are useful to treat more similar compounds as a single entity, even if they are placed in different regions of the graph.

In the following paragraphs are summarized the various steps of a Group Calibration.

9.4.2 Define Groups in the Method Panel

To set up a Group Calibration, you should have already set up a normal Calibration, that is you should have already defined a *Schedule* (a set of Standard and Sample Chromatograms) and a *Method*. See Sections 9.1.1 and 9.1.2.

To define the Named Groups, execute the following steps:

1. Switch to the *Method Panel* in the main window; the upper table (labeled `Blob recognition`) shows the already recognized Blob IDs; the lower table, labeled `Groups`, is initially empty.

2. Click on the button `add`, immediately below the Groups table: a window showing a *list of all Blob IDs* will be displayed; the window assigns a temporary name to the Group being defined, that is `Group` followed by an increasing number (the name could be later changed by the user).

3. Select two or more Blob IDs, holding the CTRL key; the selection should be done according to analytical criteria; in the example, we just selected compounds having similar names.

   Press `Ok`; a new row is inserted in the Group table, showing the new Group; the row displays the following information:

   - a Group ID (that is a sequential identifier, assigned by the system);
   - the Group Name (which is initially assigned by default as the word `Group` followed by the ID number);
   - the list of the Blob IDs belonging to the Group.

4. When one or more groups have been defined, it is possible to verify whether a given Blob ID is included in one or more Groups, by simply moving the mouse on the upper table; when the mouse passes over a Blob ID belonging to some Group, the list of the Groups is shown as a tooltip.

5. It is possible to change the name of the Group (assigning a more significant term) by directly writing inside the cell table.

6. The operation can be repeated for more Groups.

7. At any time, the user can delete one or more groups through the button `delete`; subsequent operations of adding and deleting groups will destroy the regular sequence of ID numbers: this however is not a problem, since IDs are just identifiers, and not counters.

8. At the end, the user must *save* the Method before continuing with the calibration and other operations.

Important Note: the definition of Named Groups is an activity that must be performed after the Blob recognition; the two activities cannot be mixed, in order to avoid complicated side effects. For this reason, the Blob ID structure should not be changed once one or more Groups have been defined. If the user tries to delete a Blob ID when Groups are present, a message will be displayed, warning the user that the action will reset the Group structure.

9.4.3 Integrate All Chromatograms

After that the Method has been saved, it is possible to integrate the whole set of chromatograms included in the schedule through the menu command `Action|Integrate All Chromatograms` [2].

Once the integration has been completed, you can view the traditional Results Table, clicking on the tab `Data Analysis` of the lower pane:

If the current Method defines Named Groups, a new tab (page), named `Grouping` will appear in the lower pane, at the right of Data Analysis. By clicking on it, a new table, with the results of the integration of Groups, will be shown.

This table reports a row for each Group defined in the Method, and 5 columns:

1. in the *first* column, the *ID* of the Group, as defined in the Method;
2. in the *second* column, the *Name* of the Group, as defined in the Method;
3. in the *third* column, the *Area* of the Group, calculated as the sum of the areas of the Blob IDs which constitute the Group;
4. in the *fourth* column, the *Area Percent* of the Group, calculated as the sum of the areas percent of the Blob IDs which constitute the Group;
5. in the *fifth* column, the *Concentration* of the Group (empty at the moment; will be calculated after that Group Calibration will be done).

By comparing the former two figures, it is easy to verify, for example, that the area of the Group Tyr-Cat (5672969.2) is the sum of the areas of the three Blob Ids which constitute the Group (3592113.7, 592520.2, 136974.5, 461300.2, 890060.6).

9.4.4 Group Calibration

If the current Method defines Named Groups, two new menu items appear in the Action Menu: `Action|Create Calibration Group` and `Action|Show Calibration Group`. These commands perform actions similar to `Action|Create Calibration`

[2]Note that if the schedule had been previously integrated with a different Method – for example, with a similar Method not having Named Groups – the software will warn that the current Method is different from the Method stored in the Results files, and will ask if you which Method should be used for the integration; the correct answer in this case is to preserve the Method file, since we are applying a new Method.

and `Action|Show Calibration` commands, with reference to Groups rather than to Blobs.

To perform a Calibration of the schedule using Groups rather than Blobs, execute the following steps.

1. Execute the menu command `Action|Create Calibration Group`.
2. An *informative window* with a summary of the calculations will be shown; give `Ok` to close the window.
3. The software will display the *calibration results*, opening a new window, named `Calibration Group Data`, with two pages, respectively named `Calculation` and `Graphs`.

   The *Calculation page* reports the calculation details of the Calibration.

   The *Graphs page* lets the user to view the calibration curve for each Group. The list in the upper-left corner of the windows reports all the Groups of the Method: by double-clicking on one of the groups of the list, the corresponding graph is shown in the right side, whereas the details of the calibration line and other statistical parameters are shown in the lower table.
4. The command `Action|Show Calibration Group` allows the user to view the former windows without perform again all calculations.

9.4.5 Group Quantitation

The last step of Group Calibration is the *Group Quantitation* of the unknown samples.

There is no specific command to achieve Group Quantitation for a given sample: this is automatically performed together with normal Quantitation if a Group Calibration has been previously executed on the current schedule.

The results of Group Quantitation are shown in the last column of Grouping panel.

Chapter 10

Statistics Tool

The Statistics Tool is a special tool designed to perform a series of calculations and statistical analysis on a set of chromatograms already analyzed.

To open the Statistics Tool, use the Main Menu command |[Tools][Statistics].

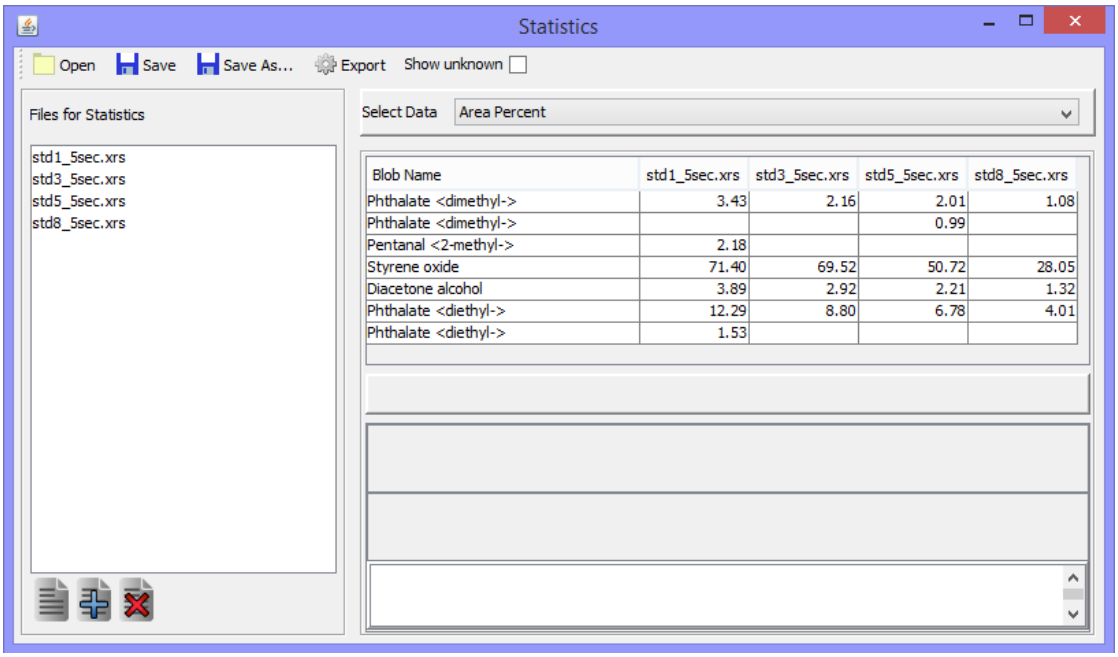


Figure 10.1: The Main Window of the Statistics Tool

10.1 Files handled by the Statistic Tool

The Statistics Tool operates on the Result Set ("`.xrs`") files generated by $Chrom^{square}$ analysis: these files contain in fact all analytical information.

Generally, several files are taken into account for a Statistics calculation. The set of these files (possibly integrated with supplementary information supplied during the Statistical Analysis) is called a *Statistics Set*.

The Statistic Set can be saved in a *Statistics Set File*, which is an XML file (suffix "`.xml`") containing all necessary information to perform the statistics calculations.

The Statistics Set File may be used for a subsequent statistics analysis, without having to reload every single "`.xrs`" file.

It is also possible to export the whole Statistics Set on a "`.csv`" file[1], so that the user could perform his own statistical calculations.

10.2 The Main Window of the Statistics Tool

A sample of the Statistics main window is shown in Figure 10.1.

The window contains the following objects:

- The `Toolbar`.
- The `File List` Panel.
- The `Data Selection` Combo-box
- The `Data` Table.

10.2.1 The Toolbar

The Toolbar contains the following commands:

- Button `Open`.
- Button `Save`.
- Button `Save as...`

[1] CSV is the acronym of "comma separated values", a standard export format that is easily handled by the most common spreadsheet processors, like Microsoft Excel.

- Button `Export`
- Check-box `Show Unknown`.

10.2.2 The File List Panel

The File List Panel is placed on the left side of the window. The Panel includes:

- The list of files: this is the list of all already loaded "`.xrs`" files, which compound the set. This is not a plain list, but is an object with *drag-and-drop* features: this means that it is possible to populate the list simply dragging one or more files from a graphic folder (like Windows "My Computer").
- The Button `Create an Empty List`  . The button is used to clear the existing list, starting a new Statistics set from scratch.
- The Button `Add Entries`  . The button is used to add one or more files to the list in the traditional way (that is opening a File Selection Dialog Box).
- The Button `Remove selected Entries`  . The button is used to delete one or more files from the list.

10.2.3 The Data Selection combo-box

The combo-box is placed on the upper-right part of the window, above the Table.

It is used to select which data should be displayed in the Table below. They are:

- All Blob data of numeric type (that is, all data displayed in the Data Analysis Result Table susceptible of numerical analysis; they include Area, Retention Time and so on).
- A new Blob datum, called *Amount*, which is normally assigned by the user using this window. It corresponds to the amount of the Blob compound for each Standard.

- A new Blob datum, called *Recovery*, which is normally calculated by the software after that the Amounts have been assigned.

10.2.4 The Data Table

The Table is built inthe following way:

- The *first column* always contains a *Blob Name*.
- Subsequent *columns* represent the various *chromatograms*, as they are being loaded: column 2 represents the first loaded chromatogram, column 3 the second one, and so on.
- The *rows* represent the various *Blobs*, displaying the data currently selected through the Data Selection combo-box.

The Data table grows (and shrinks) dynamically, as long as chromatograms are added to (or removed from) the list. Due to the different number of Blobs in various chromatograms, even the list of Blobs in column 1 changes dynamically:

- when the first chromatogram is added, column 1 is filled with all the Blobs of this chromatograms;
- when a new chromatogram is added, column 1 is redrawn, listing the previous Blobs plus the Blobs contained in the second chromatogram that were not included in the first one;
- the process is repeated each time a new chromatogram is inserted in the list; a similar process happens when chromatograms are removed.

In other words, the list of Blobs in column 1 is the *union* of all Blobs of all loaded chromatograms.

Note that the *Data Table is read-only*, with the exception of the data *Amount*, which should be compiled by the user.

10.2.4.1 Compiling the Amount Table

To compile the Data Amount Table the user should select the data through the combo-box, and then write the numerical values of the amounts of the various Blob compounds in the corresponding chromatogram columns.

The first time that Amount is selected, the table shows all cells empty. The next times, it will show the assigned values.

To make easier the compilation of large tables, a *context menu* with the *Fill down* and *Clear column* items has been set up, like in other parts of the software (see 7.2.5.4 for a detailed description of these facilities).

A sample of an empty Amount Table with the context menu opened is shown in Figure 10.2

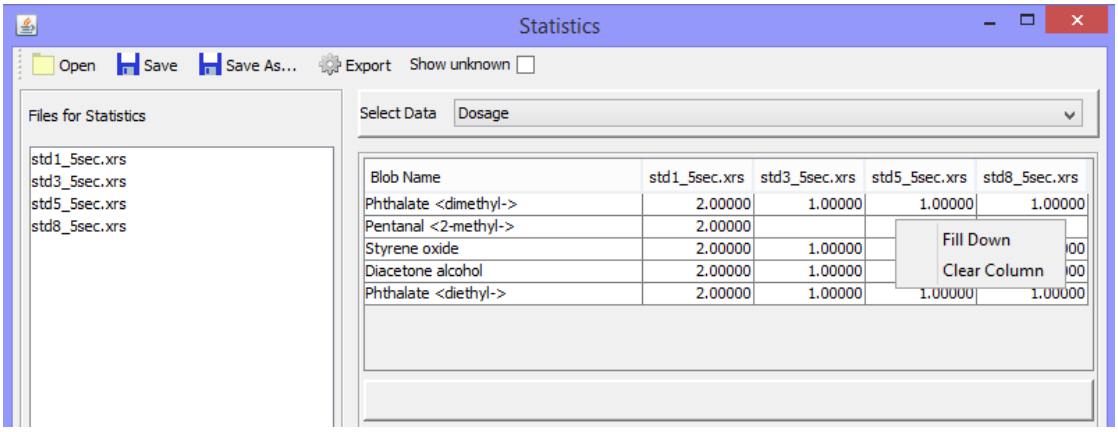


Figure 10.2: The Data Table with "Amount" selected

10.2.4.2 Statistical Analysis of Blob Data

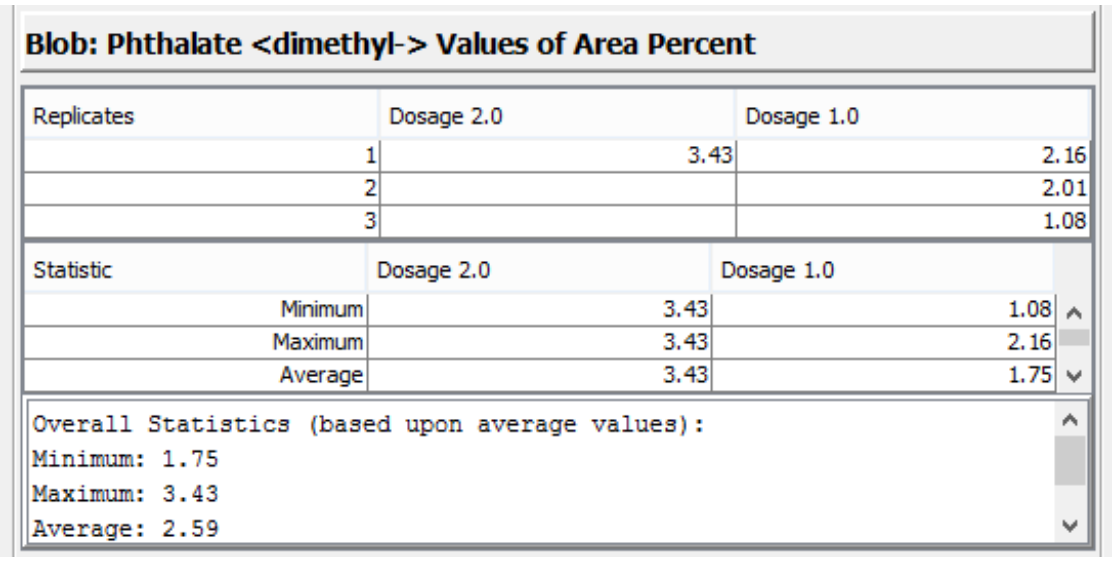
Once the Amounts Table has been compiled, it is possible to perform a statistical analysis on any Data of any Blob.

To perform this:

- Select the Data through the combo-box.
- Click with the right mouse button on the Blob row (check that row contains some values: if there is only one value the statistics do not make much sense).
- The software puts together the Blob data with the corresponding amounts, and builds a Dose/Replicates table on which performs the statistical calculations.
- A new window, reporting the statistics, is displayed.
- The user may change the current Data or the current Blob without closing the window: it contents is updated accordingly.

A sample of Statistical Analysis in the case of three Standards with one Replicate (that is three Standards with different Amount values) is shown in Figure 10.3.

A sample of Statistical Analysis in the case of three Standards with two Replicates (that is six Standards, three different Amount values, two Replicated each) is shown in Figure 10.4.



Blob: Phthalate <dimethyl-> Values of Area Percent

Replicates	Dosage 2.0	Dosage 1.0
1	3.43	2.16
2		2.01
3		1.08

Statistic	Dosage 2.0	Dosage 1.0
Minimum	3.43	1.08
Maximum	3.43	2.16
Average	3.43	1.75

```
Overall Statistics (based upon average values):
Minimum: 1.75
Maximum: 3.43
Average: 2.59
```

Figure 10.3: The Statistics Results Window: case of 1 Replicate per Standard

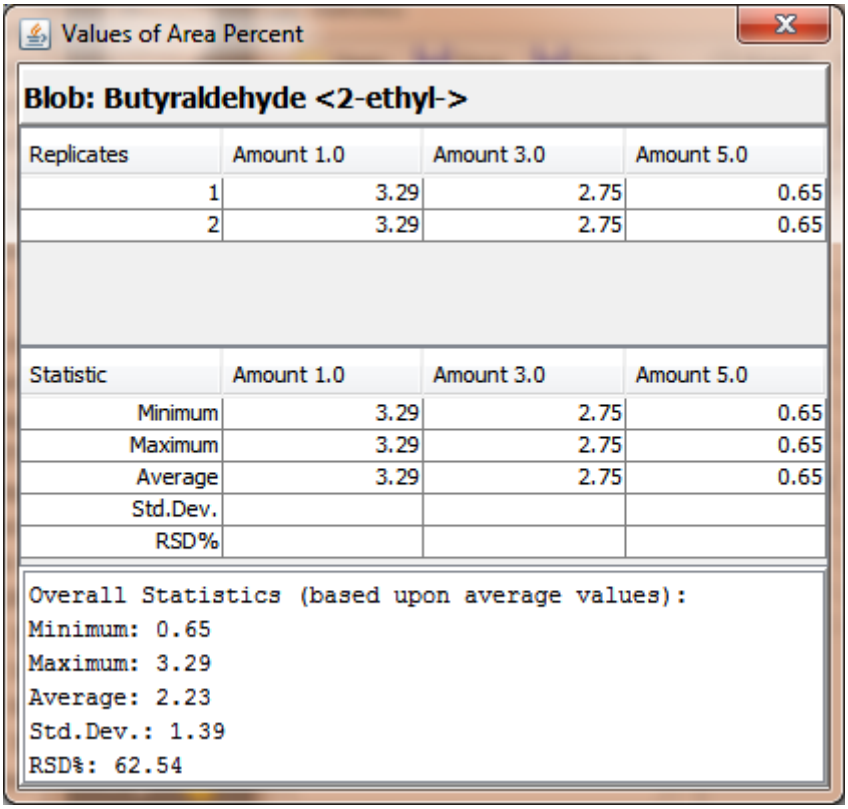


Figure 10.4: The Statistics Results Window: case of 2 Replicates per Standard

10.2.4.3 Exporting the Statistics Set

Once the Statistics Set has been completed and saved, is also possible to export it in a form suitable by further analysis bu means of other statistical packages.

To export the current Statistics Set:

- Click the button `Export`.
- A new window will open, showing all Blob Data.
- Check the boxes of the data that you want to export; use the lower buttons to select or deselect all check boxes.
- At the end, press the button `Ok`. A confirmation dialog will be shown.
- Press `Ok` again. A File Selection Dialog Box will appear, allowing to select the file, which should have the suffix "`.csv`".

Figure 10.5 shows the Select Data to Export Window.

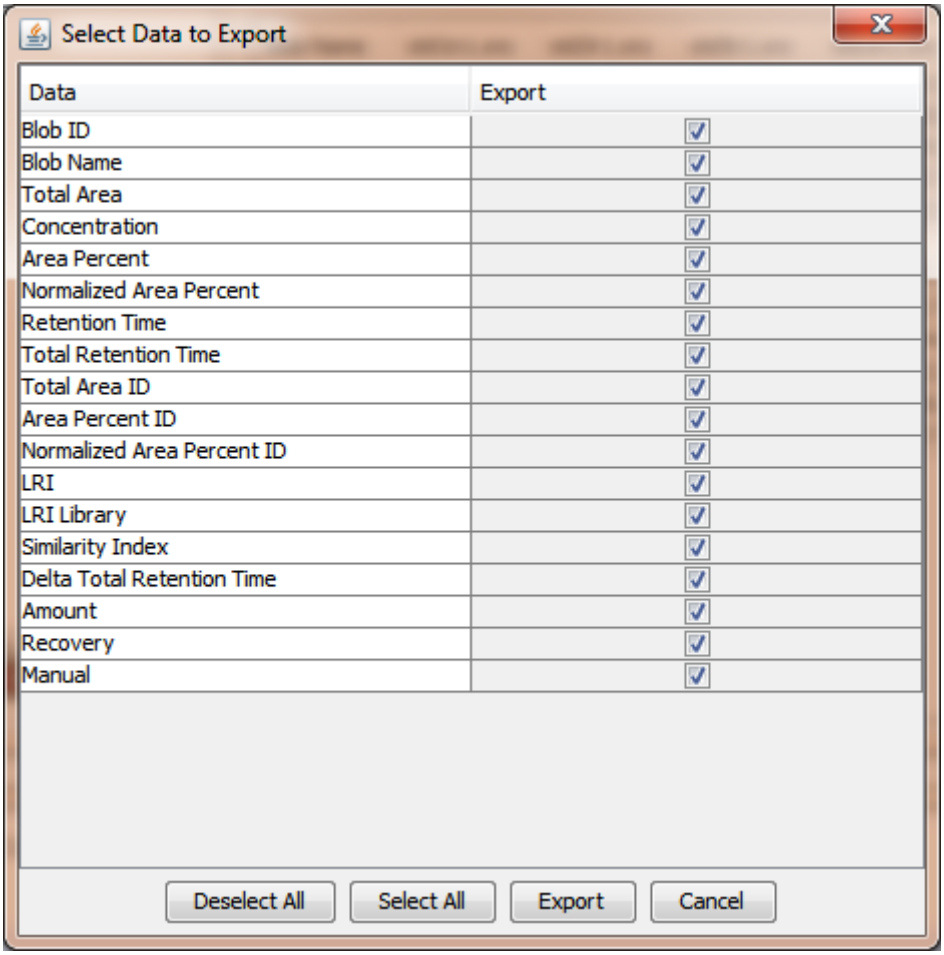


Figure 10.5: Select Data to Export Window

10.3 Operation

The steps of a typical Statistical Analysis on a set of analyzed chromatograms may be summarized as follows:

1. Load the chromatograms using the drag and drop facility of the File List Panel.

2. Check the table as long as it is being filled; change the currently displayed data using the *Data Selection combo-box*.

3. When the loading is complete, select through the combo-box the data *Amount* and compile it.

4. Assign a name to the current Statistics Set and save it on a file, using the `Save As` button. This is always recommended, since it allows to save the assigned values of the Amount Table.

5. Perform the desired statistical analysis, selecting Blobs and Data.

6. Export the Set for a further analysis.

Chapter 11

Advanced Topics

11.1 3D View

11.1.1 Description

Chrom^{square} includes a 3D facility which allows a full 3D representation of the chromatograms, which is displayed in a separate *pop up window*.

To generate this representation the user must:

- *select* a rectangular area on the 2D Map Plot (see Section 6.2.2 for how to select an area);
- give the menu command `View | 3D View`.
- (optionally) use the buttons of the 3D tool bar to modify the representation and save it as an image.

The process to create the 3D scene can take several seconds according to the performance of the computer. On a last generation PC it should take a few seconds.

The initial view is seen from the top and the orientation is the same as the view selected in the map plot of chromatogram view, as shown in 11.1.

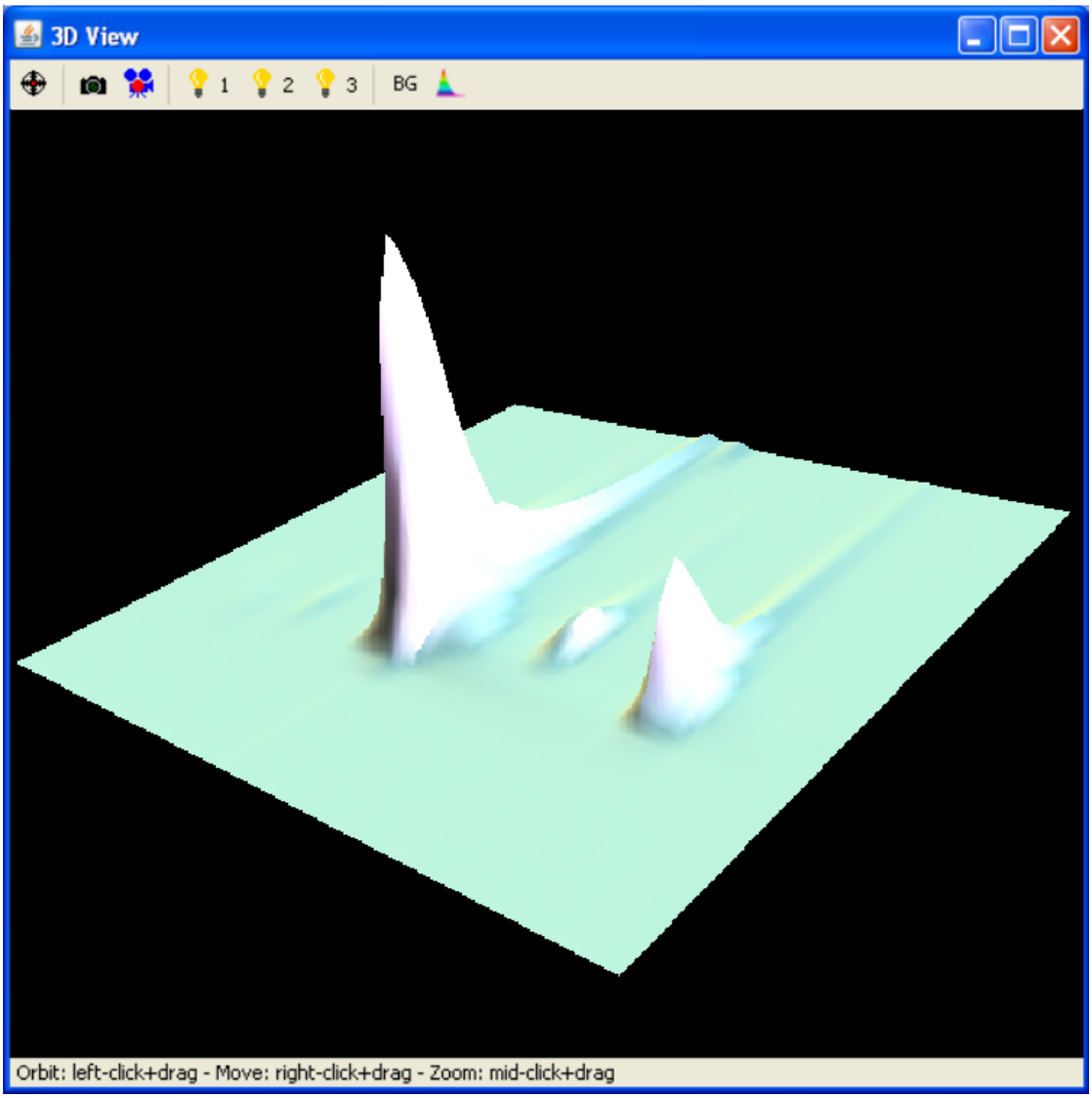


Figure 11.1: 3D View Window - Initial View

11.1.2 Changing the scene

The possible behaviours of the scene are obtained clicking and dragging the mouse in the scene.

- **Orbit** (rotates the plot according to mouse movement) is obtained by *left clicking* the mouse button and *moving* it.
- **Move** (pans the plot) is obtained by *right clicking* the mouse and *moving* it.
- **Zoom** (increases or decreases the observer's distance) is made by clicking the *central mouse button* and *moving* it; for two button mouses, the central button is generally simulated by clicking simultaneously the left and the right ones; in wheel mouses third button event is generated clicking the wheel. For most of the wheel mouses, zoom can also be obtained *rotating the mouse wheel*[1].

The Status Bar at the bottom of the window reports some short hints about how to change the scene with the mouse.

11.1.3 The 3D View Tool Bar

The 3D View window is equipped with a Tool Bar which allows to reset the scene to the initial position and to take a snapshot of the current view.

The Tool Bar contains the following controls:

- Icon  immediately *restores* the initial position for the surface.
- Icon  takes a *snapshot* of the current view. The first time that it is pressed, a File Selection Dialog Boxis shown, allowing the user to choose path and file name where to save the image. Once selected, the path and the first part of the file name will be the same for the whole session (until the 3D View window is closed), and every time the camera button is pressed a new picture will be automatically taken and saved on a file having the same prefix file name, followed by a progressive number.

[1] For additional information please refer to the specific documentation provided together with the mouse.

- Icon  takes a *movie* of the scene capturing one frame every half second. Once the user has started the recording session, the icon changes into  ; the same button allows the user to stop the recording session (the icon changes to the original one).

- Icons (actually three icons, numbered 1, 2, 3) *switch* On and Off a corresponding *directional light* that enlighten the scene.

- Icon  displays a Color Selection Dialog Box which allows to select the color for the *background*.

- Icon  *switches* On or Off the *colorization* of the surface depending on the *elevation*, that is the *intensity*. See Figure 11.2.

- `Combo Box Shininess` allows to change the *shininess*, i.e. the intensity of the *shining* of the image, selecting among a series of values (low, medium, high and default).

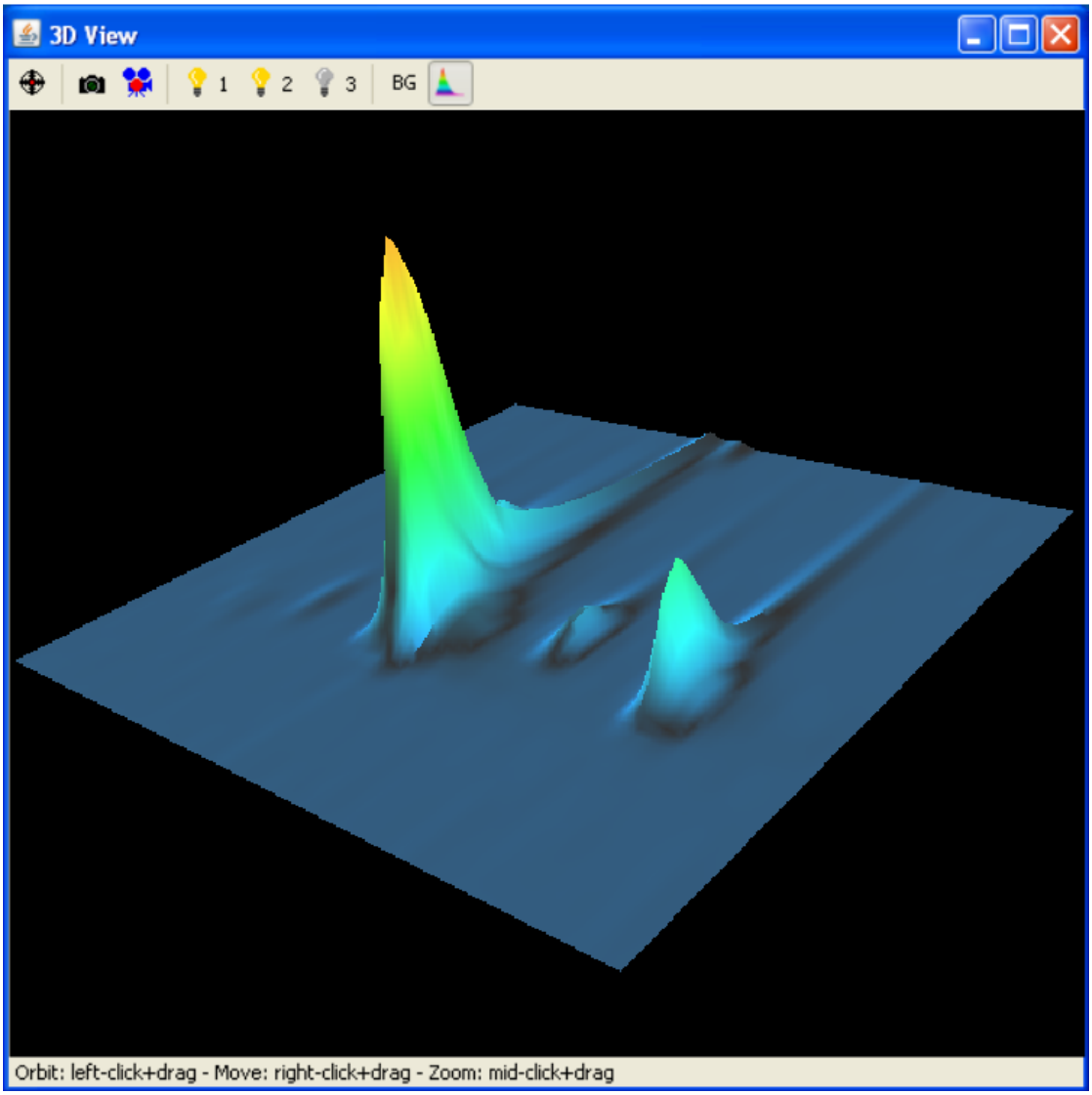


Figure 11.2: 3D View Window - Surface colorization

11.2 Compare/subtract tool

11.2.1 Introduction

The `Compare/Subtract` tool is available through the menu item `Tools | Compare Subtract Chromatograms`; it can be used even if no data is currently loaded.

This tool allows the user to *load* two different chromatograms, to *superimpose* them and to *subtract* them.

Note that the tools handles the chromatograms as *whole linear chromatograms*, i.e. without taking into account the modulations. The chromatogram Plots, therefore, represent Total Retention Time along the horizontal axis, and Intensity along the vertical axis.

11.2.2 Definitions

- `Base Chromatogram` is the first chromatogram: it determines the time and modulation boundaries and the allowed sampling rate.
- `Reference Chromatogram` is the second one. The only restraint is that the *sampling rate must be the same of the first chromatogram*: if it is not the case, no operation is obviously possible.

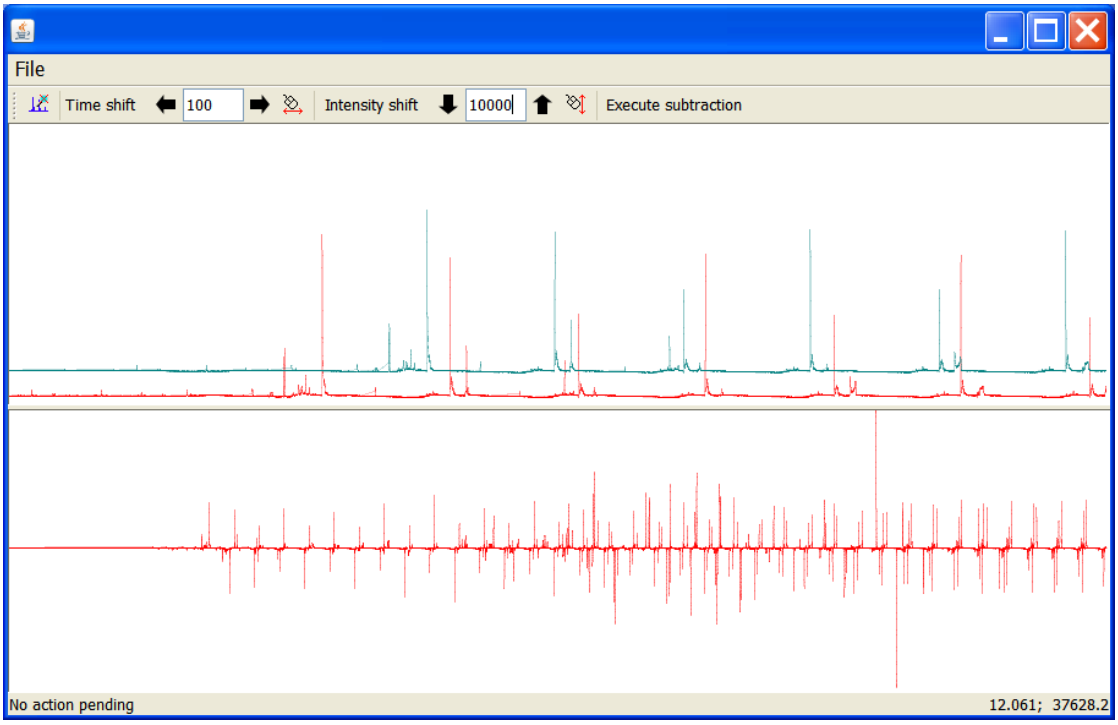


Figure 11.3: Compare/Subtract Tool

11.2.3 Description of the Compare/Subtract window

The `Compare/Subtract` window (shown in Figure 11.3) contains:

- a `File` menu;
- a `Tool Bar` with various icons and controls, shown in Figure 11.4;
- an `Upper Panel`, where will be plotted the Base Chromatogram (in *red* color), and the Reference chromatogram (in *green* color);
- a `Lower Panel`, where will be plotted the Result Chromatogram (in *red* color);
- a `Status Bar`, which reports continuously the coordinates of the mouse pointer in the Upper Panel.

11.2.3.1 Zoom

Both upper and lower Plot Panels initially show chromatograms at the maximum extent: the user may however perform *zoom* operations on the panels, in order to view more details.

Zoom operations are managed in the traditional way, dragging the mouse with the left button pressed, and releasing it when the desired region has been selected. See 6.2.3 for a general description of the zoom operations.

Zoom may be applied repeatedly, so that any desired enlargement may be achieved on both Panels.

Use the first icon of the Tool Bar (11.2.3.3) to *reset* the Zoom in both Panels and go back to the original representation of the chromatograms.

Zoom is managed separately for each panel; the reset command however applies to both panel at the same time.

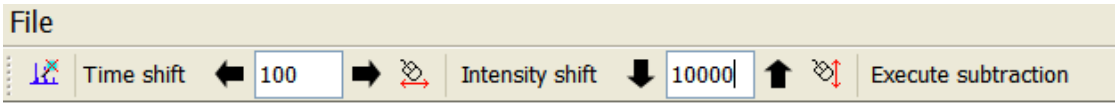


Figure 11.4: The Compare/Subtract Window Tool Bar

11.2.3.2 The Menu File of the Compare/Subtract window

The menu File contains the following items:

- `Open Base Chromatogram`. The command opens a File Selection Dialog Boxfor the choice of an existing chromatogram, which will become the *Base Chromatogram*.
- `Open Reference Chromatogram`. The command opens a File Selection Dialog Boxfor the choice of an existing chromatogram, which will become the *Reference Chromatogram*.
- `Save Result Chromatogram As`. The command opens a File Selection Dialog Boxfor the definition of a new file name, where will be possibly saved the Chromatogram arising from the subtraction operation in progress.
- `Exit`. The command closes the tool window.

11.2.3.3 The Tool Bar of the Compare/Subtract window

The Tool Bar is made up of 4 parts (divided by a thin vertical lines).

- The *first* part contains just one item:

   The command *resets the Zoom* on both panels, restoring the original chromatogram view at the maximum extent.

- The *second* part regards the *Time Shift*, that is the shifting of one chromatogram with respect to the other one along the *horizontal* axis; it includes *two horizontal arrows*, a *text field* and a *mouse icon*:

   The command shifts the reference chromatogram *left* along the time axis by a number of data points indicated in the text field.

   The command shifts the reference chromatogram *right* along the time axis by a number of data points indicated in the text fiels.

   The command allows the user to shift the reference chromatogram along the *t*ime axis by selecting the *start and the end positions*, by *clicking with the mouse onto them.*

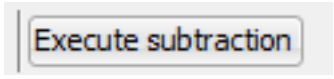
- The *third* part regards the *Intensity Shift*, that is the shifting of one chromatogram with respect to the other one along the *vertical* axis; it includes *two vertical arrows*, a *text field* and a *mouse icon*:

   The command shifts the reference chromatogram *down* along the intensity axis by an amount indicated in the text editor.

   The command shifts the reference chromatogram *up* along the intensity axis by an amount indicated in the text editor.

   The command allows the user to shift the reference chromatogram along the *intensity* axis by selecting the *start and the end positions*, by *clicking with the mouse onto them.*

- The *fourth* part just contains a button:

  The command executes the subtraction of the two chromatograms previously loaded, according to the shifts set through the Tool Bar, and displays the resulting chromatogram in the lower panel.

11.2.3.4 Shifting the reference chromatogram using the mouse

Once the user selects the button in order to shift the chromatogram upon the time or intensity axis, a prompt to select the first point is displayed in the status bar. After the start point is selected, a prompt to select the second point is displayed in the status bar. After the second click, the shift is performed.

11.2.4 Operations

A typical set of operation

1. Open the base chromatogram using menu `File | Open Base Chromatogram`.
2. Open the reference chromatogram using menu `File | Open Reference Chromatogram`.
3. Adjust the horizontal and/or vertical shifting using the Tool Bar commands (11.2.3.3) or mouse commands (11.2.3.4).
4. Press button `Execute Subtractions`.
5. Where necessary, perform some zoom operations to emphasize the details.
6. Finally save the result chromatogram using the menu `File | Save result chromatogram as`.

11.3 Compare Map Views

11.3.1 Introduction

The `Compare Map Views` tool is available through the menu item `Tools | Compare Map Views`; it can be used even if no data is currently loaded.

This tool allows the user to *load* two different chromatograms, to *view* both chromatograms as bi-dimensional Contour Maps (like in the main Map View described at 6.2, and to *combine them superimposing the two maps*, with the possibility of assigning a shift in both directions.

11.3.2 Definitions

- `Base Chromatogram` is the first chromatogram: it determines the time and modulation boundaries and the allowed sampling rate.

- `Reference Chromatogram` is the second one.

  Also in this case it is recommended that the *sampling rates* and the *modulation times* of the two chromatograms are the same. This condition is not as mandatory as in the case of comparing linear chromatograms (described at 11.2), since the combinations of the images is done on a pixel basis, so that a resulting image is always obtained. It is however important for the readability and the significance of the results.

- `Palette`: in Computer Graphics the *palette* is the set of colors used for representing the digital images. The color of each pixel is calculated through the linear interpolation of the corresponding intensity value, using a set of pairs intensity-color value. Presently the Compare Map Views tool may handle the following palettes:

  - the *Rainbow* Palette, that is the standard $Chrom^{square}$ palette used in the Map View window;
  - *4 monochromatic palettes*, that is one for each fundamental colors (*Red*, *Green*, *Blue*) plus *Black and White*.

- `Composed Map` is the map resulting from the graphical combination of the colors of the corresponding pixels in Base and Reference chromatogram Maps. The combination of colors is calculated through the technique called *Alpha Compositing*[2], which is based on an additional parameter, called *transparency* that can be set by the user through the Compare Options Window (see 11.3.3.3).

[2]In computer graphics, alpha compositing is the process of combining an image with a background to create the appearance of partial or full transparency.

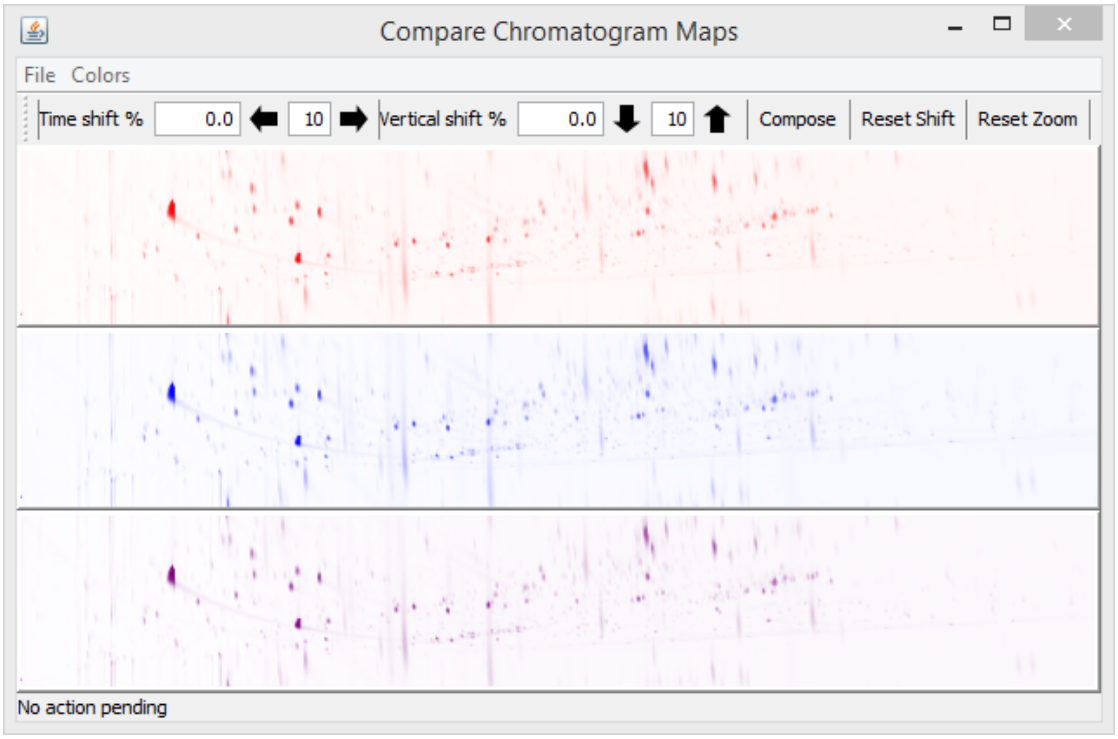


Figure 11.5: Compare Chromatogram Maps window

11.3.3 Description of the Compare Chromatogram Maps window

The `Compare Chromatogram Maps` window (shown in Figure 11.5) contains:

- a `File` menu;
- a `Tool Bar` with various icons and controls, shown in Figure 11.6;
- an `Upper Panel`, where will be plotted the Base Chromatogram Map;
- a `Central Panel`, where will be plotted the Reference Chromatogram Map;
- a `Lower Panel`, where will be plotted the Result Chromatogram, obtained by graphically combining the other two Maps;
- a `Status Bar`, which reports continuously the coordinates of the mouse pointer in the Upper Panel.

11.3.3.1 Zoom

All three Map Panels initially show chromatograms at the maximum extent: the user may however perform *zoom* operations on the panels, in order to view more details.

Zoom operations are managed in the traditional way, dragging the mouse with the left button pressed, and releasing it when the desired region has been selected. See 6.2.3 for a general description of the zoom operations.

Zoom may be applied repeatedly, so that any desired enlargement may be achieved on all Panels.

Use the `Reset Zoom` button to clear the zoom and restore the Map views at the maximum extent.

Both Zoom and Reset Zoom commands act contemporaneously on all panels.

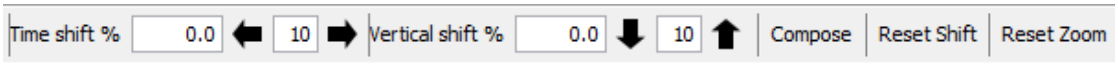


Figure 11.6: The Compare Chromatogram Maps Window Tool Bar

11.3.3.2 The Menu File of the Compare Chromatogram Maps window

The menu File contains the following items:

- `Open Base Chromatogram`. The command opens a File Selection Dialog Boxfor the choice of an existing chromatogram, which will become the *Base Chromatogram*.
- `Open Reference Chromatogram`. The command opens a File Selection Dialog Boxfor the choice of an existing chromatogram, which will become the *Reference Chromatogram*.
- `Reload last configuration`. The command automatically opens the last chromatograms loaded in a previous session.
- `Exit`. The command closes the tool window.

11.3.3.3 The Menu Colors of the Compare Chromatogram Maps window

The menu Colors contains just one item:

- `Options`. The command opens a new window, which allows the user to set the colors of the various chromatogram maps. The window is shown in Figure 11.7 and contains the following items:

  Palette for Base Chromatogram
  This is a combo-box for the selection of the Graphical Palette to be used for the representation of the Base Chromatogram.

  Check Box Dark Background (for Base Chromatogram
  When the check box is *set*, the interpolation of the colors is computed in such a way that lower intensities correspond to darker colors (the effect is to have *bright blobs on a dark background*); when the check box is *unset*, the interpolation of the colors is computed in such a way that lower intensities correspond to brighter colors (the effect is to have *dark blobs on a white background*).

  Palette for Reference Chromatogram
  This is a combo-box for the selection of the Graphical Palette to be used for the representation of the Reference Chromatogram.

  Check Box Dark Background (for Reference Chromatogram
  The operation is the same of the check box described above.

  Text Field Transparency
  This is the parameter for Alpha Compositing, expressed as a decimal value ranging from 0 to 100 0, which indicates *"how much" of the reference chromatogram is composed with the base*: in practice, 0 means *all base*, 100 means *all reference*.

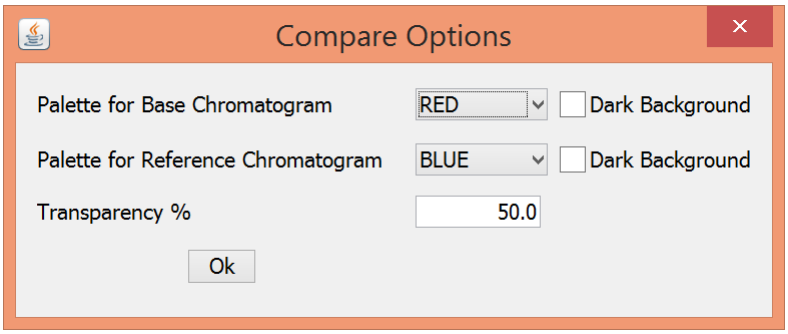
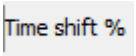


Figure 11.7: The Compare Options Window

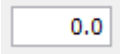
11.3.3.4 The Tool Bar of the Compare Chromatogram Maps window

The Tool Bar is made up of 5 parts (divided by thin vertical lines).

- The *first* part regards the *Time Shift*, that is the shifting of one chromatogram map with respect to the other one along the *horizontal* axis; it includes the following items:



Time Shift section identifier.



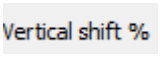
A text field which shows the current Time Shift.

The left arrow is the command which *left shifts* the current Time Shift by the amount specified in the text field between the two arrows.

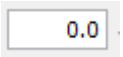
A text field where the user can specify the Time Shift amount interval that will be applied for each pressing of the two adjacent arrow commands.

The right arrow is the command which *right shifts* the current Time Shift by the amount specified in the text field between the two arrows.

- The *second* part regards the *Vertical Shift*, that is the shifting of one chromatogram map with respect to the other one along the *vertical* axis; it includes the following items:



Vertical Shift section identifier.



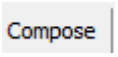
A text field which shows the current Vertical Shift.

The up arrow is the command which *up shifts* the current Verytical Shift by the amount specified in the text field between the two arrows.

A text field where the user can specify the Vertical Shift amount interval that will be applied for each pressing of the two adjacent arrow commands.

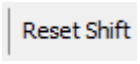
The down arrow is the command which *down shifts* the current Vertical Shift by the amount specified in the text field between the two arrows.

- The *third* part contains a button:

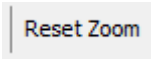
  The command executes the composition of the two chromatograms currently loaded, according to the shifts set through the Tool Bar and, and displays the resulting chromatogram in the lower panel.

- The *fourth* part contains a button:

  The command reset the Time Shift and the Vertical Shift, restoring the original view.

- The *fifth* part contains a button:

  The command reset the Zoom for all panels.

The amounts of all shift operations (both for time shifts and for vertical shifts) are always represented as percentage of the overall size of the plot in the shift direction.

11.3.4 Operations

A typical set of operation is as follows:

1. Open the base chromatogram using menu `File | Open Base Chromatogram`.
2. Open the reference chromatogram using menu `File | Open Reference Chromatogram`.
3. Adjust the horizontal and/or vertical shifting using the Tool Bar commands. (11.3.3.4).
4. Press button `Compose`.
5. Where necessary, perform some zoom operations to emphasize the details.

11.4 Define Polygonal Blobs

It is possible to identify one or more Blobs creating a polygonal region. Every Blob whose top is included in that region gets the name and the ID defined together with the region itself.

The region is saved into the Method so that every chromatogram processed with that Method inherits the region settings.

To define such region:

1. Select the page `Data Analysis` in the lower panel of Chromatogram view, then check the *check box* `Polygon mode`.
2. Left click repeatedly the mouse onto the Map Plot to select the vertices of the polygon. The area is automatically closed, so there is no need to re-select the first point. Right click of the mouse deletes the last point inserted, so undo is possible.
3. When finished, uncheck the check box `Polygon mode`: a prompt for the name of the Blob is displayed.

Figure 11.8 shows a sample of a polygonal region being defined.

Figure 11.9 shows the assignment of the name to the defined region.

Figure 11.10 shows the integration results with the identification of the previously assigned polygonal Blob ID.

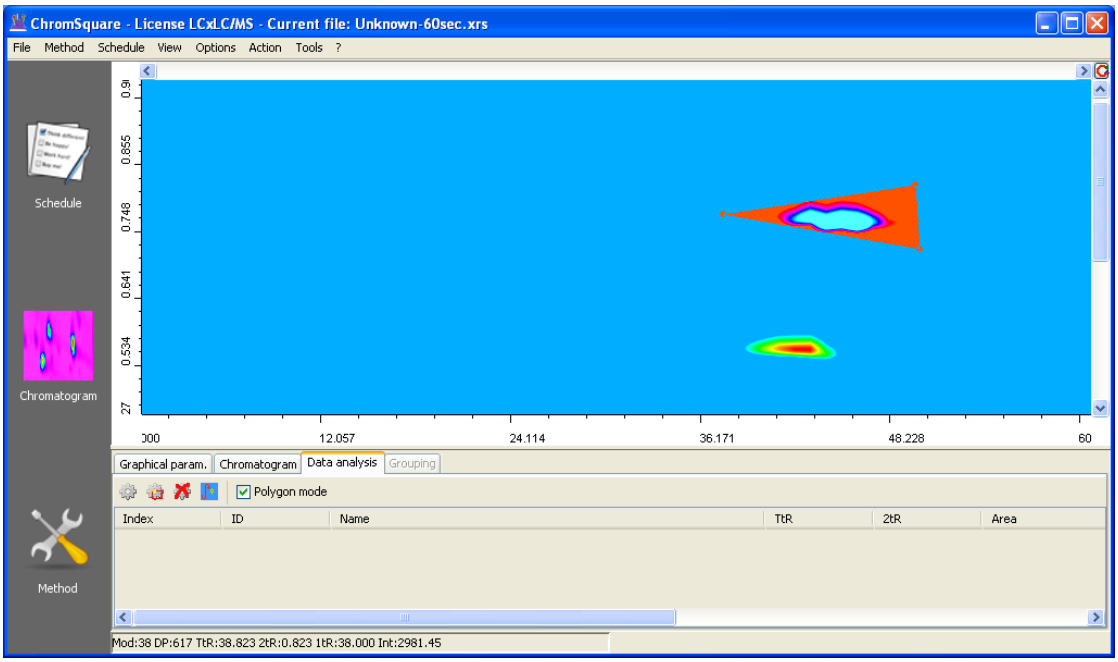


Figure 11.8: Defining a Polygonal Region

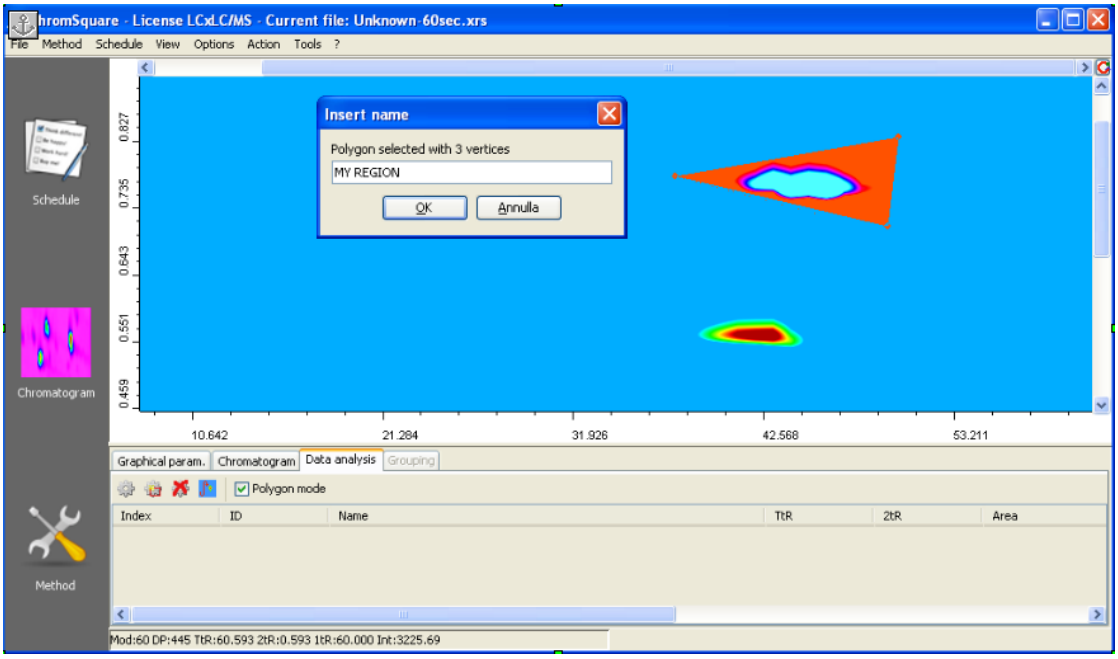


Figure 11.9: Entering the polygon region name

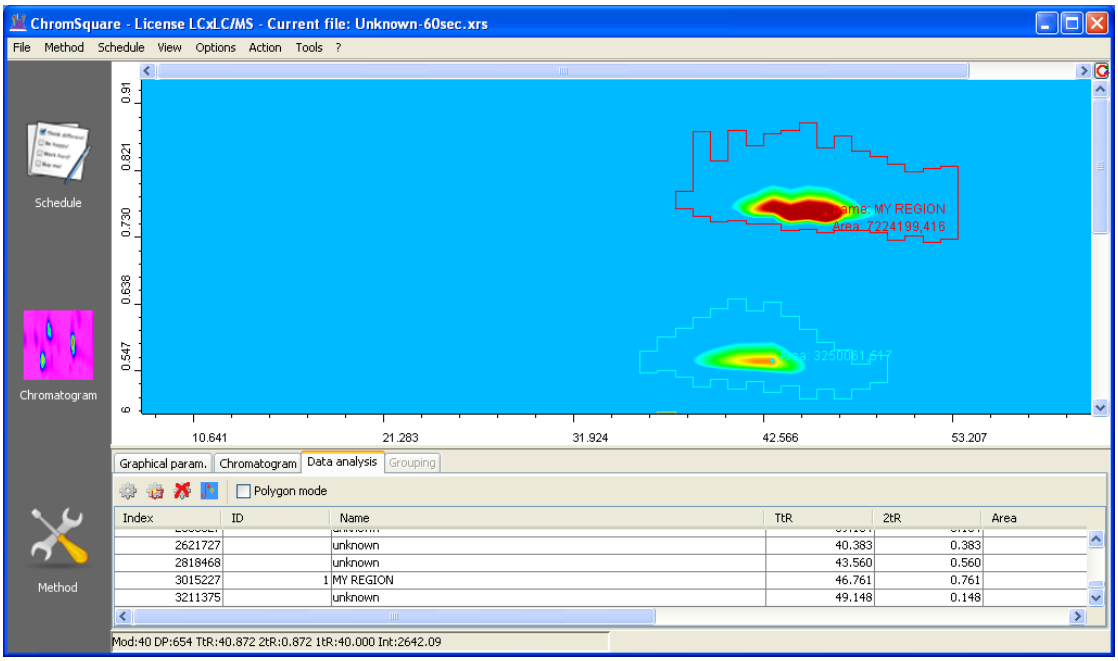


Figure 11.10: The polygonal Blob after integration

Note.

The selection for the blob is not intended to be a manual recognition. The region that has been selected is stored in the method and it is used for further integrations for any chromatogram that is analyzed using that certain method. In fact, it is equivalent to the time-band recognition for a linear chromatogram. The difference is that in the Blob case the chromatogram is modulated, and therefore the selected rectangle must contain at least one whole modulation on the x-axis (that means that the blob must have the maximum only in that modulation) and a certain number of data points (or an interval of time, that is the same) in the 2nd dimension, according to the repeatability of Retention Times in the analysis.

The time-based blob recognition is always made upon the top, regardless the extent of the contour of the Blob. Naturally if the rectangle involves more than one modulation, the Blob can have the top in any of the modulations that have been selected. If there are two or more areas overlapping, the top of the Blob is assigned to the region that has the nearest barycenter.

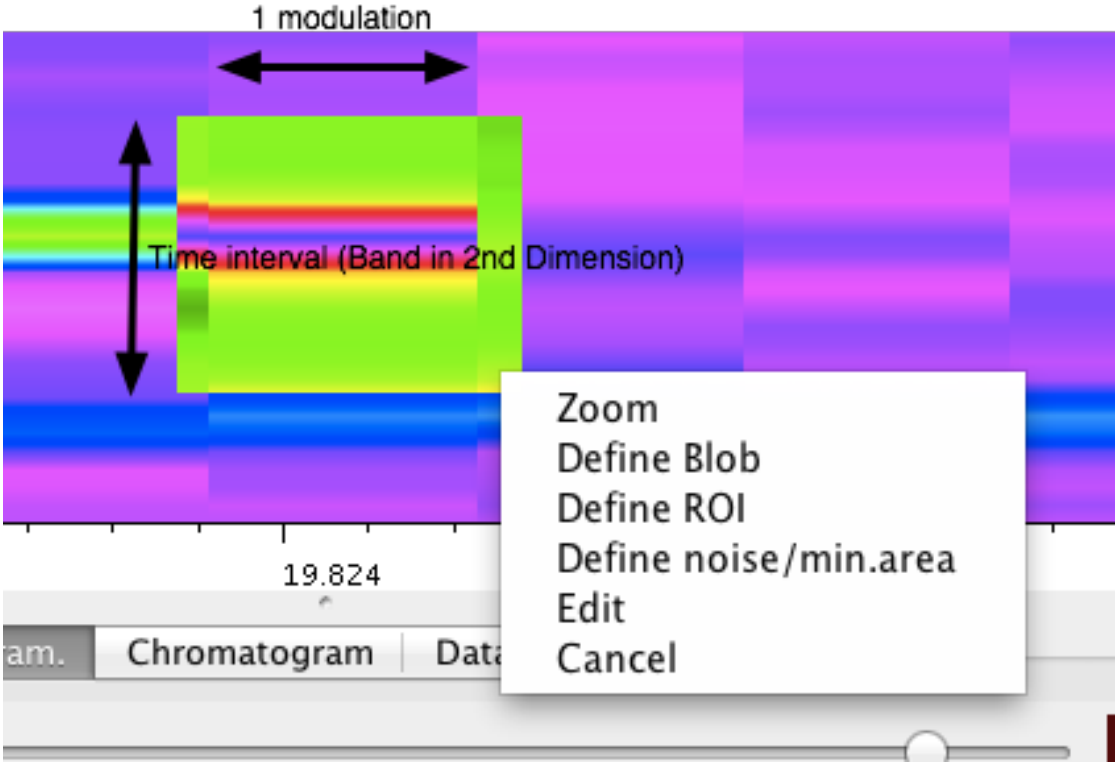


Figure 11.11: Details of polygonal Blob definition

11.5 Report Template Editor

The Report Template Editor allows the user to prepare a custom Report Template. This template will be automatically added to the list of Report Templates and appear in the selection panel when the command `Action | Generate Chromatogram Report` is executed.

The Report Template Editor window is shown in Figure 11.12

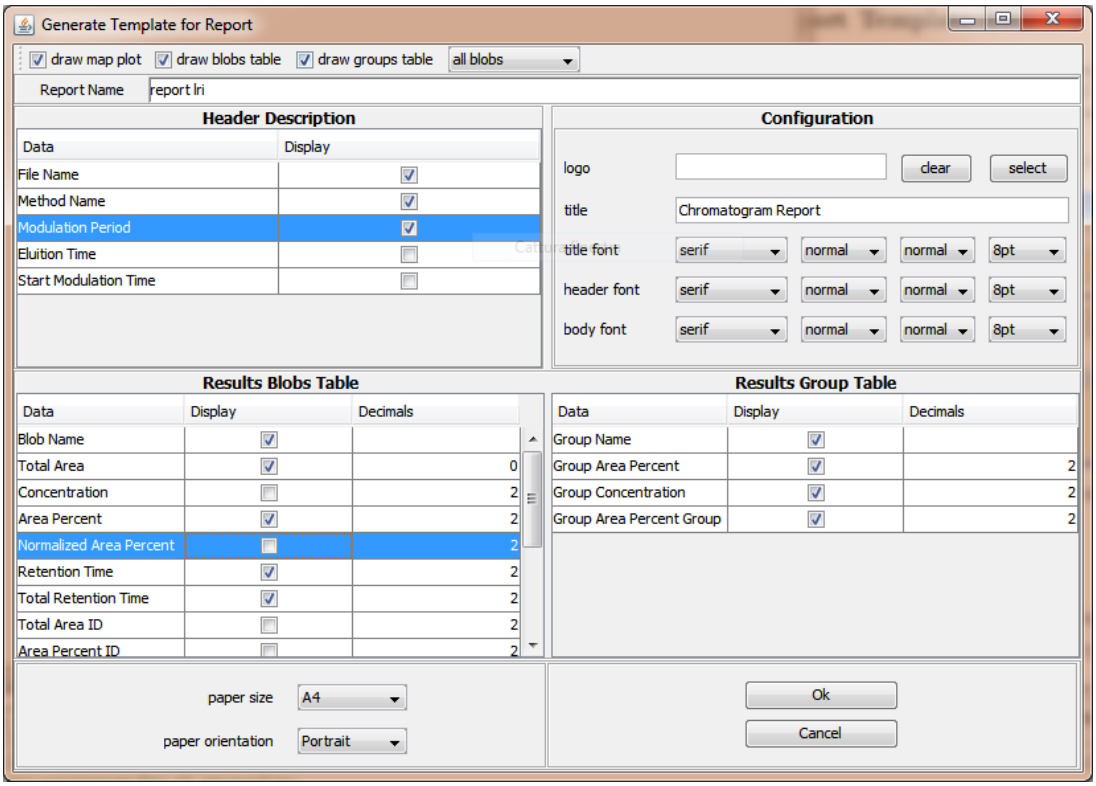


Figure 11.12: The Report Template Editor Window

The window contains various panels, each one dedicated to a particular aspect of the report.

The Report may contain 4 sections: Report Header, Map Plot, Blobs Table, Groups Table. All sections, except the Report Header, are optional and may be selected through the check-boxes of the Upper Panel.

11.5.1 Upper Panel

This panel contains the Toolbar and the Report Name.

11.5.1.1 The Report Template Editor Toolbar

The toolbar contains:

- Check-box `draw map plot`: when checked, the report will include a Map Plot of the current chromatogram. The Map will be drawn in the first page of the Report and will correspond to the image currently displayed in the Map Plot window.
- Check-box `draw blobs table`: when checked, the report will include the table of Blobs, that is the table displayed in the Data Analysis window (see 6.5.1). The main features of the table may be then customized in the Results Blobs Table Panel (see 11.5.4).
- Check-box `draw groups table`: when checked, the report will include the table of Groups, that is the table displayed in the Grouping page (see 6.6). The main features of the table may be then customized in the Results Groups Table Panel (see 11.5.5).
- Combo-box `all blobs`: it allows to select between the representation of all Blobs or of only the identified ones.

11.5.1.2 Report Name

The report name is the identifier of the current Template. To create a new Template, enter a name in the text field. If a template with this name already exists, it will appear in *red*: in this case the existing template will be modified.

11.5.2 Header Description Panel

It contains 5 check-boxes for the customization of the Report Header. They establish if the following information should be displayed or not in the Report Header:

- File Name
- Method Name

- Modulation Period
- Elution Time
- Start Modulation Time

11.5.3 Configuration Panel

This Panel contains various types of customization regarding the whole Report. It includes:

- The `Logo` sub-panel: a set of controls for the selection of a graphic file containing a *logo* that will be displayed in the Report Header.
- The `Title` that will be displayed in the Report Header.
- The `Font` sub-panel. It contains a set of combo-boxes which allow to customizes the fonts used in various parts of the report, namely:
  - The title font. It is the font used in the Report Header.
  - The header font. It is the font used for the Headers of the Tables.
  - The body font. It is the font used for the normal text.

  For each kind of font, the user can then customize:
  - The *font family* (serif, sans-serif, cursive, fantasy, monospace).
  - The *font type* (normal, italic).
  - The *font weight* (normal, bold).
  - The `fonts size`, expressed in *points*.

11.5.4 Results Blobs Table Panel

This is a table composed of 3 columns and as many rows as the columns of the Blobs Table displayed in the Data Analysis window (see 6.5.1).

The function of this table is to select which columns of the Results Table, that is which Blob data, have to be printed and, in the case of numerical values, how many decimal digits are requested[3].

[3]This customization is important since not all columns could be reported in a readable form on a printed report (unlike the Blobs Table on the screen, where there are scrolling bars and the other facilities reported in section 6.5.2)

The first column (Data) specifies the datum name; the second one (Display) contains a check-box for displaying the datum or not; the third one a combo-box for the choice of the number of decimal digits to be used for that datum.

11.5.5 Results Groups Table Panel

This table has the same functions of the preceding one (11.5.4), applied in this case to the Group Table displayed in the Grouping Page (6.6).

The operations are exactly the same as those described for the Blobs Table.

11.5.6 Lower Panel

This last panel contains:

- A combo-box for the selection of `paper size` (*A4* or *Letter*).
- A combo-box for the selection of `paper orientation` (*portrait* or *landscape*);
- The button `Ok`, for closing the window saving the current template.
- The button `Cancel`, for closing the window without saving.

11.6 Profiles

11.6.1 Description

Profiles are sets of information concerning the data files; profiles have been introduced in $Chrom^{square}$ software to simplify user input; in fact:

- data files are not always self-explaining, and in many cases further information must be supplied by the user;
- in many cases, in laboratory routine, the characteristics of data files being analyzed do not change very often; in these cases, information specific to the type of data file could be fully specified only the first time that this file is used; for all other instances, information could be given as a *link* to some sort of already defined set of information. We call *profiles* these set of information.

Profiles are defined and maintained by the user; all profiles are stored in a specific XML file, `ChromaConfig.xml`, which is resident in a reserved folder. A sample of `ChromaConfig.xml` is automatically generated during installation.

Important note: users must not directly modify the `ChromaConfig.xml` file; only the software should do this; in the case of improper modifications, the entire profile engine could not work properly any more. If this happens, the best thing to do is to reinstall the software from scratch (it is also possible to manually delete the file, and manually generate all user-defined profiles).

Profiles change with the kind of data files being used. Not all data files are presently linked to a profile. The working scheme is however equal for all kind of profiles.

11.6.2 Defining a new Profile

This Section reports the various steps that the user must execute to define a New Profile.

1. Give the `File Open` command; a File Selection Dialog Boxwill be opened.
2. Browse to the desired folder and *Select* a data file; the present example points out the case of GCsolution or GCMSsolution files acquired in "`.gcd`" or "`.qgd`" data formats.
3. In the the upper-right side of the Dialog Box a combo box named `Profile` will be shown, together with the two buttons `New` and `Remove` (see Figure 11.13).
4. Press the `New` button; a new Dialog Box, named `New Profile`, will open.
5. Assign a name for the new profile; fill all other information requests displayed on the `New Profile` Window.
6. Press `Save`; this will close the window, returning to File Open Dialog Box.
7. 5. In the Profile section of the File Open Dialog Box, use the combo box to select the profile just generated. Note that the combo box only shows the profile types that are compatible with the selected data file and software license.

The new profile is permanently stored in the `ChromaConfig.xml` file and can be used when a similar data file should be analyzed.

The button `Remove` of the profile section allows the user to delete from `ChromaConfig.xml` file the profiles that are no longer used.

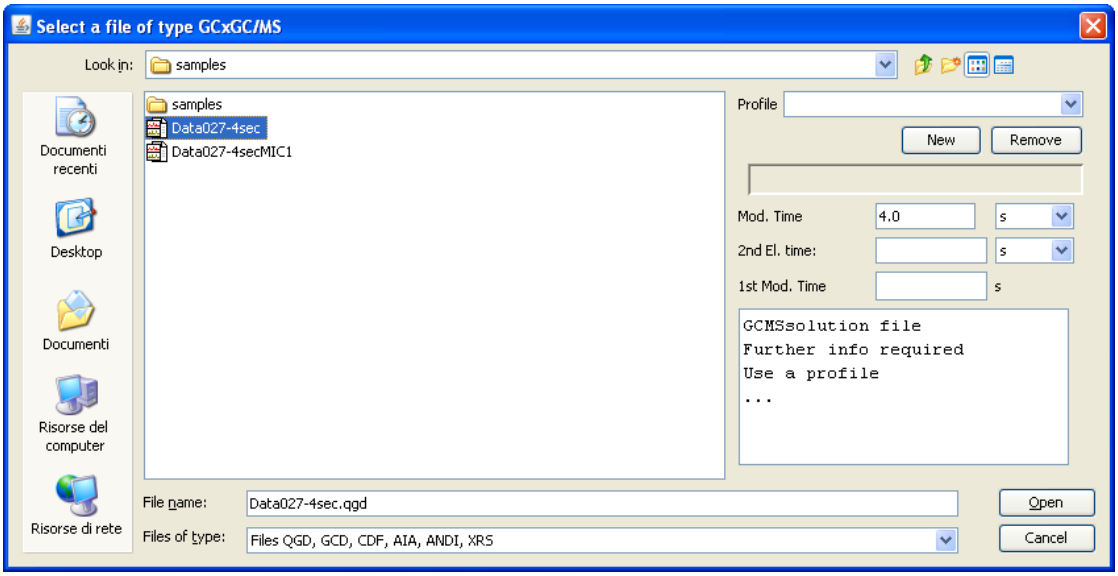


Figure 11.13: Open Dialog Box with Profile Combo Box

11.6.3 Profiles for GCsolution or GCMSsolution files acquired in ".gcd" or ".qgd" data formats

In the case of GCsolution or GCMSsolution files acquired in ".gcd" or ".qgd" data formatsthe Profile window allows the user to select among the different *types of data* that are included in the data file, as shown in Figure 11.14.

The first operation is the choice of the *type of data*, using the radio-buttons located in the left part of the window; presently the following types are provided:

1. Shimadzu GCMSsolution TIC.
2. Shimadzu GCMSsolution SCAN.
3. Shimadzu GCMSsolution SIM.
4. Shimadzu GCMSsolution MRM.

For the SCAN, SIM and MRM data type, also a *Group number* should be specified. This is an integer starting from 1, that corresponds to the particular group that should be extracted and analyzed.

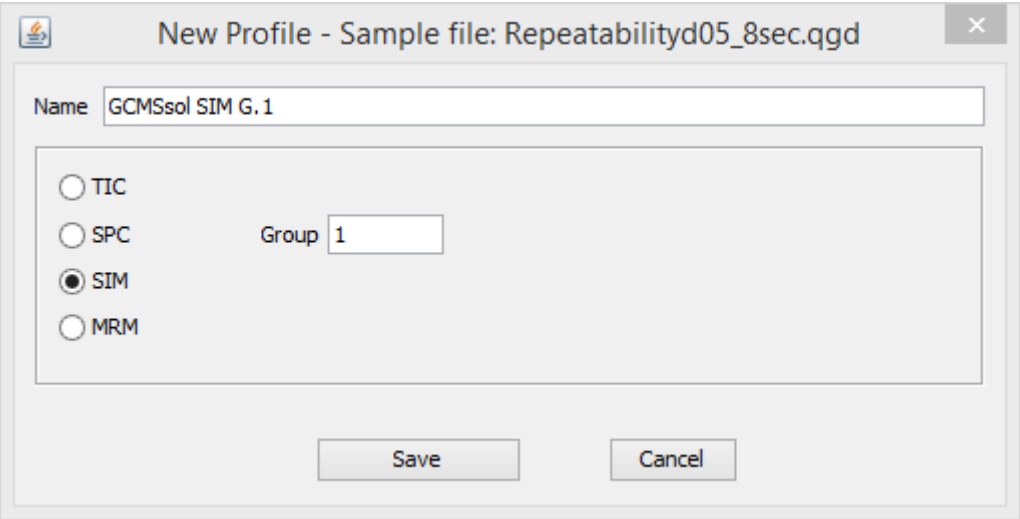


Figure 11.14: The New Profile window for GCMS case

11.7 Ion selection

11.7.1 Description

If the chromatogram under analysis is generated by a mass spectrometer, a selection of fragments can be used instead of the whole intensity. This procedure is called *Ion Selection*.

Ion Selection may be used in two cases:

- In *Quantitative Analysis*, where a different Ion Selection may be defined for each Blob ID: Quantitative Analysis will be based on Blob areas calculated summing only the contribution of the selected fragments.
- In *Map Plot*, where the visualization of the Blobs will take into account only the selected fragments.

Ion selection follow the following rules:

- Ion Selections are *independent*: that is, the user can define a different selection for each Blob ID and for Map Plot.
- Ion Selection *features are the same for all cases*. They are described in the next section 11.7.2.

- All Ion Selection information are stored in the Method. Information are stored and retrieved as character strings, using a particular format, described in 11.7.3.

11.7.2 Ion Selection Features

Starting from Release 2.3, *Chrom*^{square}allows *Multiple Ion Selections*, having the following features:

- A *Multiple Ion Selection* includes one or more items, called *Ion Selection Entries*.
- An Ion Selection Entry corresponds to a *range* of one or more ion fragments having the same *Scan Group* and *Event Type*. The full feature list of an Ion Selection Entry includes:
  - The *Scan Group*. This is a 1-based positive integer number.
  - The *Event type*. It can be *SCAN*, *SIM* or *MRM*.
  - The *Start Ion*, that is the m/z of the First Ion of the range. It can be an integer or decimal number, according to the spectra precision specified in the Method.
  - The *End Ion*, that is the m/z of the Last Ion of the range. It can be an integer or decimal number, according to the spectra precision specified in the Method.
  - The *color* that will be used to represent the Ion Selection Entry in the Chromatogram Panel. It is an integer number which describes the RGB components of the color.

11.7.3 Ion Selection String

Ion Selection Strings are used to describe Multiple Ion Selections: this is an easy way for passing the description among the various parts of the software, and for storing and retrieving it.

Strings may be directly coded by the user, following the rules described in this section: anyway, this way may become awkward, especially in the case of Ion Selections containing several entries. The preferred way is to generate and modify Ion Selections by means of the special tool called *Ion Selection Editor*.

Ion Selection Strings are coded according to the following rules.

- Ion Selection String is a string of one or more items separated by *commas*.
- Each item corresponds to an Ion Selection Entry, which describes a range of Ion fragments. The item contains 6 fields, separated by *colons*:
  1. the character "E" (Enabled) or "D" (Disabled). This field can be used to temporary disable an item, without deleting it, so that it can be easily restored.
  2. one of the characters "S" (SCAN) or "I" (SIM) or "M" (MRM).
  3. the Group Number.
  4. the m/z of the Start Ion of the Ion Range.
  5. the m/z of the End Ion of the Ion Range.
  6. the color index assigned to the Ion Range.
- The following conventions will also apply:
  - to identify the *whole range* of Ions, set both *Start Ion and End Ion equal to 0*;
  - to identify the Ion having the *maximum intensity*, set both *Start Ion and End Ion equal to -1*.

11.7.4 Ion Selection Editor

The Ion Selection Editor is a Dialog Box which is automatically displayed in the following cases:

- when the user double-clicks on a cell of the Ion Selection column of the Blob ID Table (see 7.2.5.1); in this case the Editor allows to set up the Ion Selection relative to the selected Blob ID;
- when the user clicks on the the Ion `Selection for Map Display` button of the Method Panel; in this case the Editor allows to set up the Ion Selection used for displaying Blobs in the Map Plot;

The Ion Selection Editor is only a facility for an easy compilation of the Ion Selection String. Closing the window with the `Save` button will generate an Ion Selection String that will be saved in the Method.

A sample of the Ion Selection Editor window is shown in Figure 11.15.

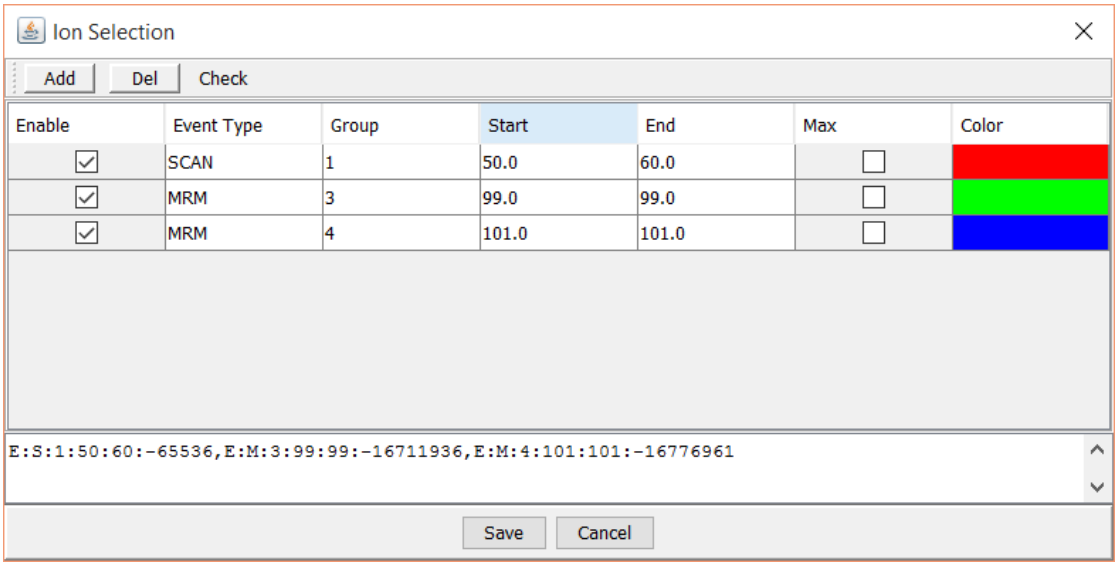


Figure 11.15: The Ion Selection Editor Dialog box

11.7.4.1 Window

The Ion Selection Editor Window includes:

- The **Toolbar** with the following three buttons:
  - **Add** Button
  - **Del** Button
  - **Check** Button
- The **Table**; it includes a *row* for each Ion Selection Entry (see 11.7.2) and the following *7 columns*, which correspond to the Ion Selection Entry features:
  1. The **Enable** check box. When selected the corresponding selection is applied, otherwise it is temporary disabled and maintained as reference for future use.
  2. The **Event Type** combo box. The possible events are **SCAN**, **SIM** and **MRM**.
  3. The **Group** edit box. The edit field is equipped with a *Spin Control* for a swift editing.
  4. The **Start** Ion edit box.
  5. The **End** Ion edit box.

6. The **Max** check box: when selected, the option for selecting the Ion having the *maximum intensity* (that is Start and End Ions equal to -1) is automatically assigned.
7. The **Color** button: when pressed, a standard window for the selection of a Color is displayed (see 11.16)

- A **Text Area**, used for showing the Ion Selection String corresponding to the table currently displayed.
- The button **Save**.
- The button **Cancel**.

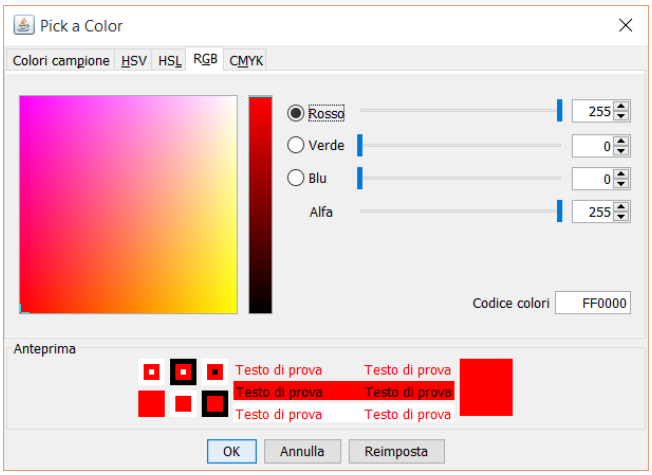


Figure 11.16: The Color Editor Dialog box

11.7.4.2 Operation

The user can populate and manage the table according to the following instructions:

- Click on the `Add` button to insert a new row; the row will be shown, with the various fields set to default values.
- Compile the various fields. Check the compatibility among the values that are being assigned (a formal check is done by the software, by this control cannot cover all possibilities: it is user's responsibility to verify that the assigned selections are meaningful).

- Click on the `Add` button again to insert other rows.
- Use the `Del` button to delete the row where the mouse pointer is currently placed. Alternatively, it is possible to disable a row using the `Enable` check box.
- At any time, use the `Check` button to control the consistency of the currently assigned values: this operation also generates the corresponding Ion Selection String, that will be shown in the Text Area in the lower part of the window.
- Click on the `Save` button to close the window accepting all current values and saving the Ion Selection String into the Method; a Check operation is always performed before actually saving, and the window is not closed if severe errors or inconsistencies are present.
- Click on the `Cancel` button to close the window without saving.

11.8 MRM Confirmation

11.8.1 Description

If the chromatogram under analysis is generated by a MRM mass spectrometer, an elaborate filter about the mass fragments can be applied. This procedure is called *MRM Confirmation*.

A different MRM Confirmation may be defined for each Blob ID.

MRM Confirmation features are described in the next section 11.8.2.

MRM Confirmation follows the following rules:

- MRM Confirmations are *independent*: that is, the user can define a different filter for each Blob ID.
- All MRM Confirmations information are stored in the Method. Information are stored and retrieved as character strings, using a particular format, described in 11.8.3.

11.8.2 MRM Confirmation Features

Starting from Release 2.3, $Chrom^{square}$allows *Multiple MRM Confirmations*, having the following features:

- A *Multiple MRM Confirmation* includes one or more items, called *MRM Confirmation Entries*.
- A MRM Confirmation Entry describes the expected ratio (and relative tolerance) between the intensities of two given MRM fragments of a given Scan Group at the top of the Blob ID.

  The full feature list of an MRM Confirmation Entry includes:

  - The *Scan Group*. This is a 1-based positive integer number.
  - *Fragment 1*: the mz of the fragment that will be used as *numerator* of the ratio.
  - *Fragment 2*: the mz of the fragment that will be used as *denominator* of the ratio.
  - The `Ratio` between the intensities of the two MRM fragments.
  - The `Tolerance` associated to the Ratio.

11.8.3 MRM Confirmation String

MRM Confirmation Strings are used to describe Multiple MRM Confirmations: this is an easy way for passing the description among the various parts of the software, and for storing and retrieving it.

Strings may be directly coded by the user, following the rules described in this section: anyway, this way may become awkward, especially in the case of MRM Confirmations containing several entries. The preferred way is to generate and modify MRM Confirmations by means of the special tool called *MRM Confirmation Editor*.

MRM Confirmation Strings are coded according to the following rules.

- MRM Confirmation String is a string of one or more items separated by *commas*.
- Each item corresponds to an MRM Confirmation Entry, which describes a range of Ion fragments. The item contains 6 fields, separated by *colons*:

  1. the character "E" (Enabled) or "D" (Disabled). This field can be used to temporary disable an item, without deleting it, so that it can be easily restored.
  2. the Group Number.

3. the m/z of the Fragment 1.
4. the m/z of the Fragment 2.
5. the Ratio.
6. the Tolerance.

11.8.4 MRM Confirmation Editor

The MRM Confirmation Editor is a Dialog Box which is automatically displayed when the user double-clicks on a cell of the MRM Confirmation column of the Blob ID Table (see 7.2.5.1): in this case the Editor allows to set up the MRM Confirmation relative to the selected Blob ID;

The MRM Confirmation Editor is only a facility for an easy compilation of the MRM Confirmation String. Closing the window with the `Save` button will generate an MRM Confirmation String that will be saved in the Method.

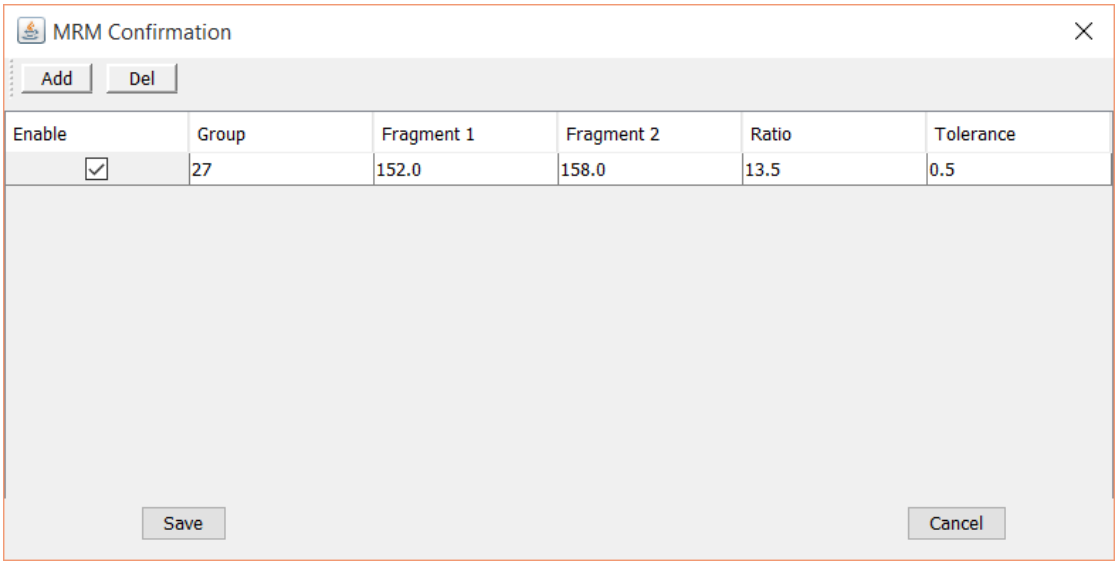


Figure 11.17: The MRM Confirmation Editor Dialog box

A sample of the MRM Confirmation Editor window is shown in Figure 11.17.

11.8.4.1 Window

The MRM Confirmation Editor Window includes:

- The **Toolbar** with the following two buttons:
  - **Add** Button
  - **Del** Button
- The **Table**; it includes a *row* for each MRM Confirmation Entry (see 11.7.2) and the following *6 columns*, which correspond to the MRM Confirmation Entry features:
  1. The **Enable** check box. When selected the corresponding selection is applied, otherwise it is temporary disabled and maintained as reference for future use.
  2. The **Group** edit box. The edit field is equipped with a *Spin Control* for a swift editing.
  3. The **Fragment 1** edit box. The edit field is equipped with a *Spin Control* for a swift editing.
  4. The **Fragment 2** edit box. The edit field is equipped with a *Spin Control* for a swift editing.
  5. The **Ratio** edit box.
  6. The **Tolerance** edit box.
- The button **Save**.
- The button **Cancel**.

11.8.4.2 Operation

The user can populate and manage the table according to the following instructions:

- Click on the `Add` button to insert a new row; the row will be shown, with the various fields set to default values.
- Compile the various fields. Check the compatibility among the values that are being assigned (a formal check is done by the software, by this control cannot cover all possibilities: it is user's responsibility to verify that the assigned selections are meaningful).
- Click on the `Add` button again to insert other rows.

- Use the `Del` button to delete the row where the mouse pointer is currently placed. Alternatively, it is possible to disable a row using the `Enable` check box.
- Click on the `Save` button to close the window accepting all current values and saving the MRM Confirmation String into the Method; a check operation is always performed before actually saving, and the window is not closed if severe errors or inconsistencies are present.
- Click on the `Cancel` button to close the window without saving.

11.9 Assembling Data Files

11.9.1 Description

This feature applies mainly to LCxGC(MS).

The feature becomes necessary when the chromatographic instruments that operate the first and second elution are managed separately, and a new injection is performed for each modulation. It is then possible to *assemble* more chromatograms into a unique data file; this feature is used when the modulations had been saved onto different files (a single file for each modulation, with files numbered as a sequence).

11.9.2 Procedure

To assemble more chromatograms, please use the following procedure:

1. Select the menu item `File | Assemble Datafile`; a File Selection Dialog Box, with the title `Select one of the files to assemble`, will appear. See Figure 11.18.
2. Select one of the files shown in the window, and press `Open`.
3. A new window (`Select files to assemble`) with two panes will be shown. The *left* pane will contain the *list of all the files having similar names* and ending with different sequence numbers; the right pane will contain the files that the user wants to assemble. See Figure 11.19.

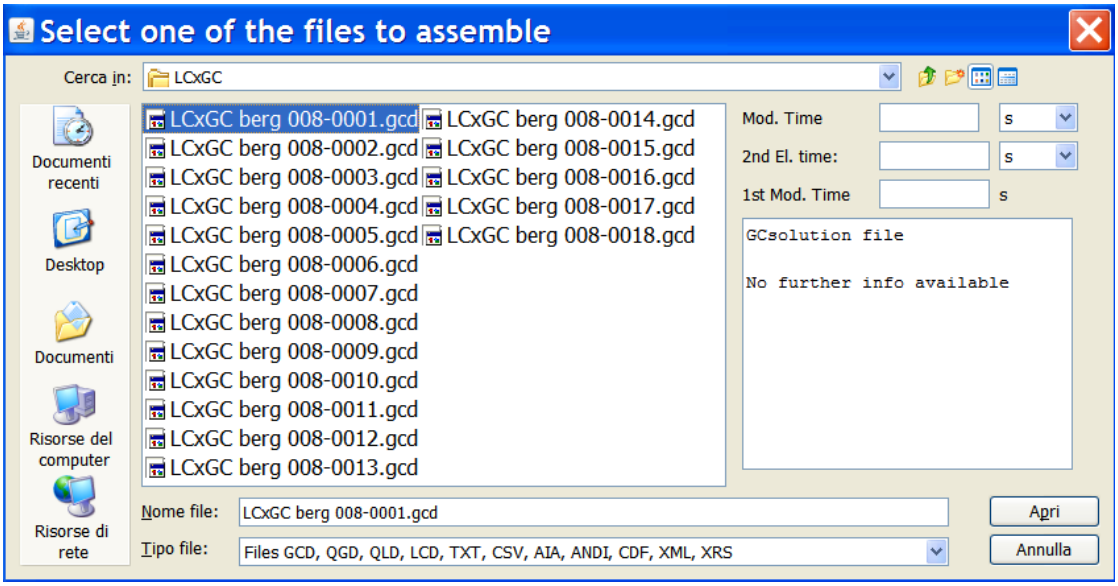


Figure 11.18: The File selection Dialog box of the Assemble case

4. Use the two central buttons marked with » and « to transfer all files between the panes, or double-click on a file name in order to transfer just one file. See 11.20.

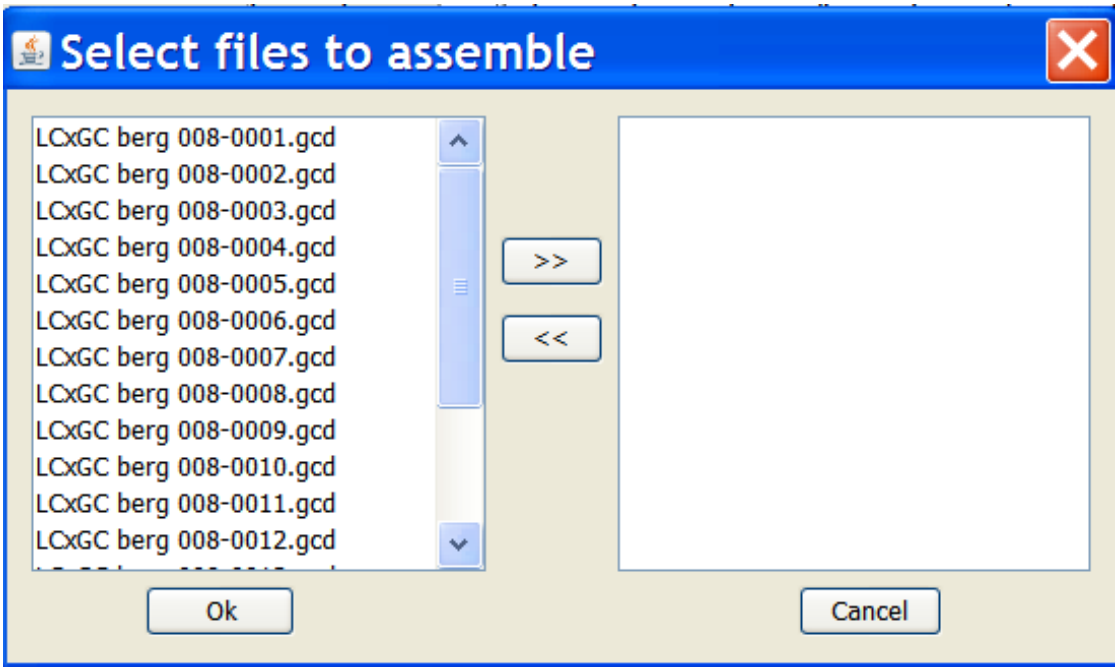


Figure 11.19: List of the files to assemble

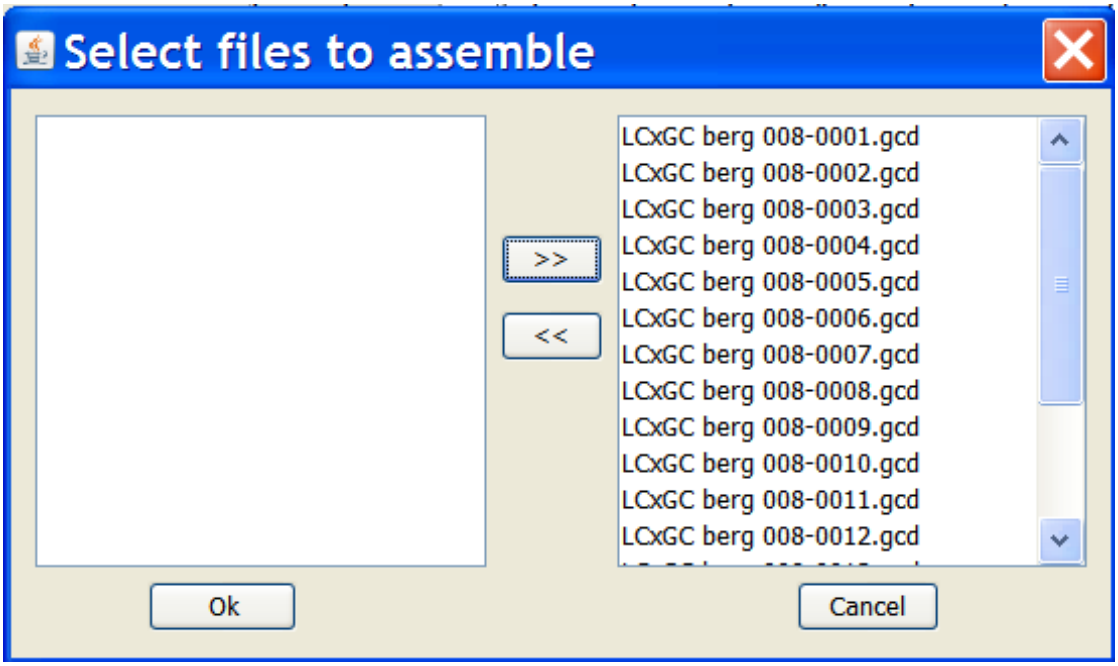


Figure 11.20: Files selected for assembling

5. Press `Ok` when the desired configuration is achieved. A new dialog box is finally shown, asking for confirmation (see Figure 11.21).

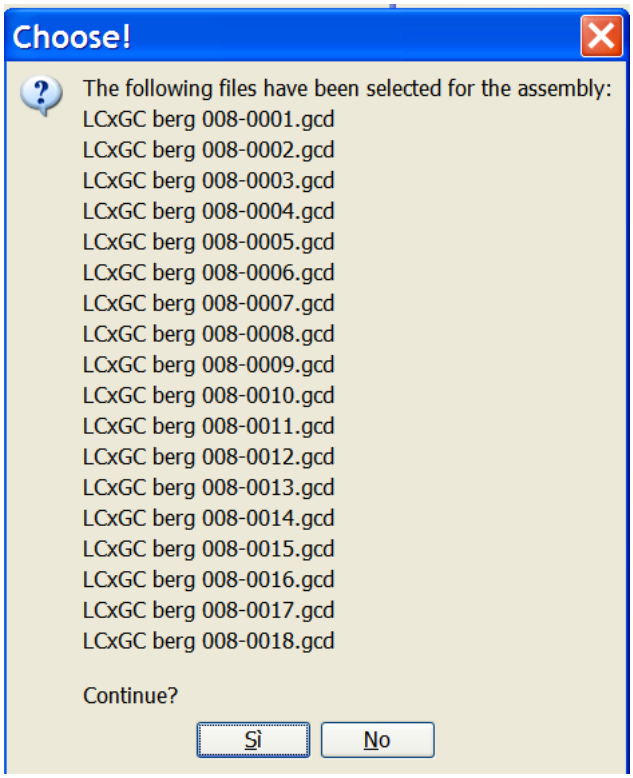


Figure 11.21: Asking for confirmation before assembling

6. The software will load all single datafiles, assembling them into an unique *Chrom*^{square} "`.cdf`" datafile, that will be saved in the same directory. The assembled datafile will have a name composed by the name of the first original datafile, followed by the specification of the modulation time (in the assembly case, the modulation time is given by the final retention time of the single datafiles, that should be the same for all files). The name of the new file is shown in a Message box, like in Figure 11.22.

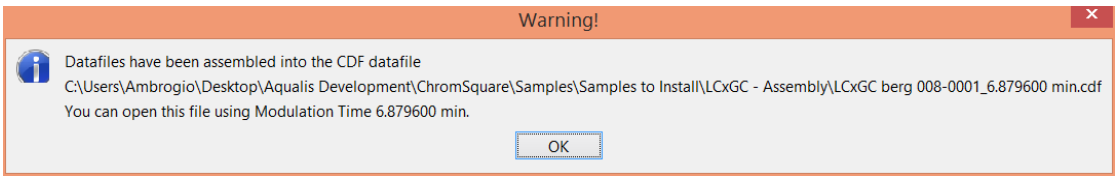


Figure 11.22: The message showing the name of the resulting assembled file

7. At the end of the procedure, the resulting assembled chromatogram is displayed (see Figure 11.23). The software will treat this chromatogram like any other chromatogram, obtained in the traditional way. The user should only consider that in this case the number of modulations is generally much lower that in the other cases: this could cause some limitations at zoom level.

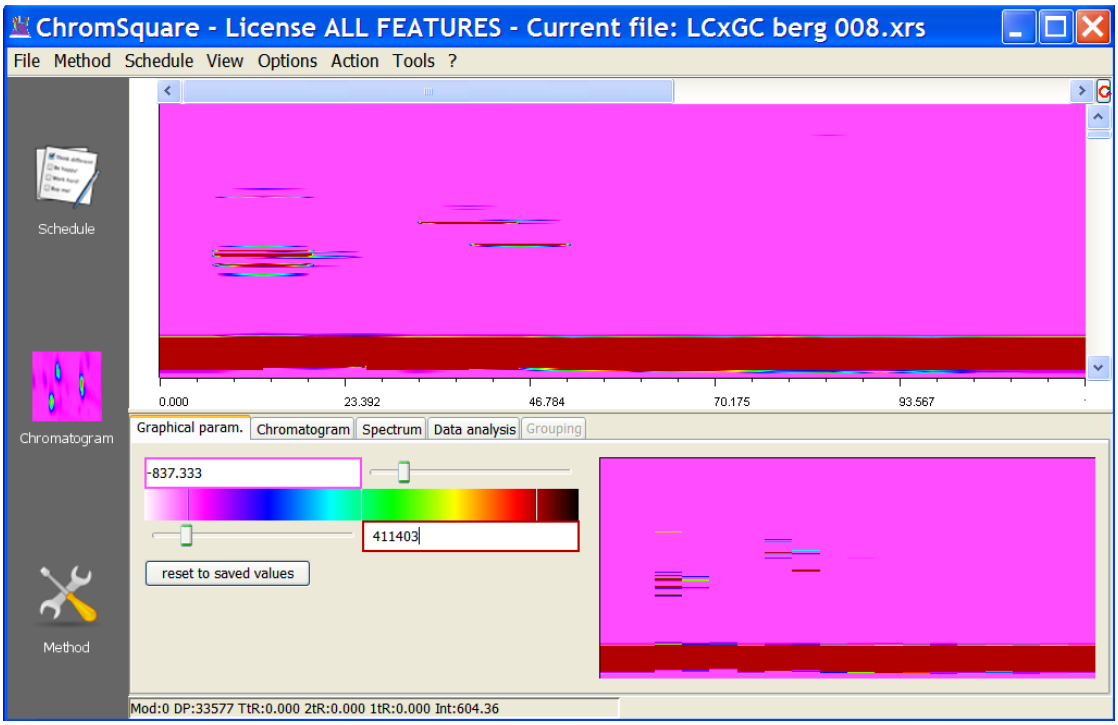


Figure 11.23: The resulting assembled chromatogram

The assembling procedure described above can be performed just once; for all next usages it is much faster to directly load the assembled CDF file: the results are exactly the same. Also the modulation time is automatically assumed, being included in the name of the datafile.

11.10 Local Chromatogram Editor

11.10.1 Calling the Local Chromatogram Editor

The *Local Chromatogram Editor* is an optional feature that can be activated or hidden through the *editor* parameter specified in the *general* section of the configuration file; when the parameter is activated a menu item *Edit* is shown in the

Map View Selection Context Menu (6.2.4). Please contact Technical Support for managing this option.

11.10.2 Visualization

The Editor window is shown in the Figure 11.24.

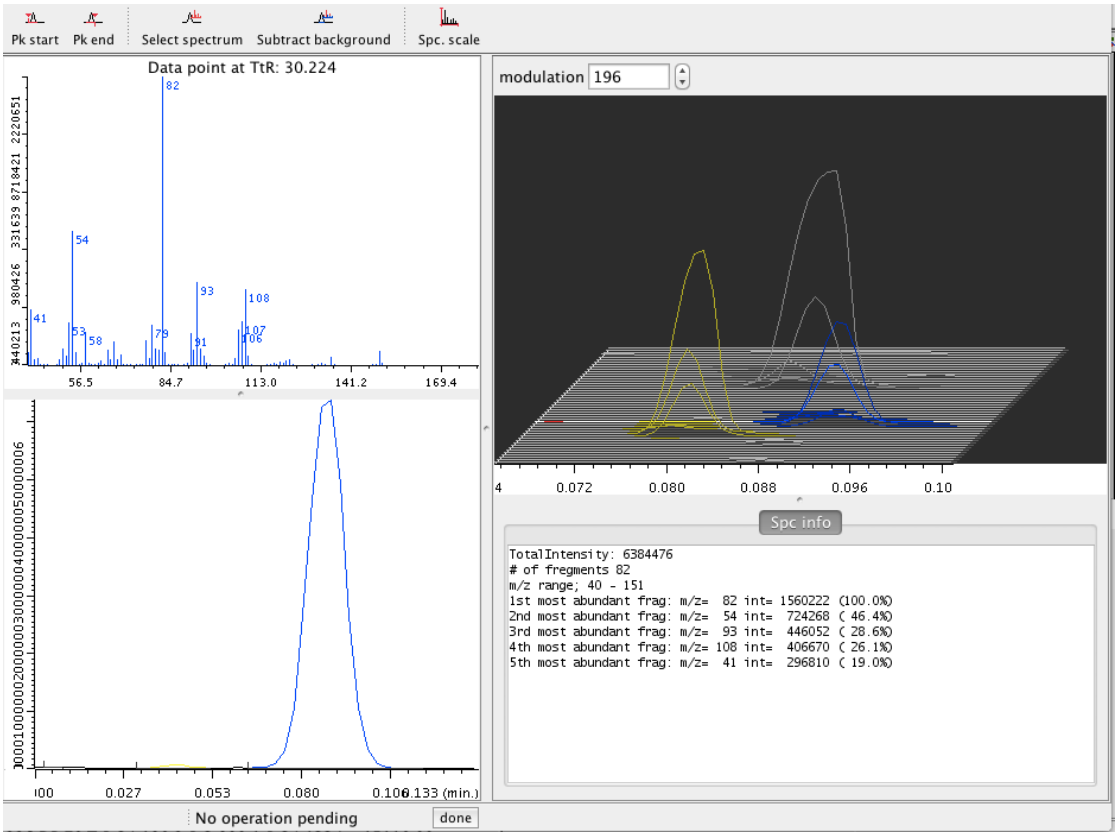


Figure 11.24: The Local Chromatogram Editor window

On **top left view** the current spectrum is displayed.

On **bottom left** view, the current modulation is shown.

On **top right view** the range of modulations selected through the rectangle in map view is displayed. The user can select current modulation using the spin control of the top of the view. If this view has the focus, the current modulation can be browsed using cursor up and down keys.

On **bottom right** view info messages are shown.

11.10.3 Operations

11.10.3.1 Pk start

The command allows to move the baseline along the chromatogram trace. Proceed as follows:

- Click on the button `Pk start`.
- The message label in the status bar shows "select peak".
- Click on the peak to correct the baseline.
- The message label in the status bar shows "select new start point".
- Click on the chromatogram trace on the point where the new baseline is intended to start.
- The baseline is moved. The message label in the status bar shows "no pending operation".

The user can abort the operation at any time pressing the button "done" near the message label in the status bar.

11.10.3.2 Pk end

The command allows to move the baseline along the chromatogram trace. Proceed as follows:

- Click on the button `Pk end`.
- The message label in the status bar shows "select peak".
- Click on the peak to correct the baseline.
- The message label in the status bar shows "select new end point".
- Click on the chromatogram trace on the point where the new baseline is intended to end.
- The baseline is moved. The message label in the status bar shows "no pending operation".

The user can abort the operation at any time pressing the button "done" near the message label in the status bar.

11.10.3.3 Select spectrum

The command allows to compute the total spectrum fc an interval of data points. Proceed as follows:

- Click on the button `select spectrum`.
- The message label in the status bar shows “select initial data point”.
- Click on the trace to select the start of the interval.
- The message label in the status bar shows “select final data point”.
- Click on the trace to select the end of the interval.
- The spectrum view shows the total spectrum instead of the punctual one; the title indicates the range, the message label in the status bar shows “no pending operation”. content...

11.10.3.4 Subtract background

The command allows to subtract the background from the spectrum selected through operation “Select spectrum”. Proceed as follows:

- Once selected a spectrum range, click on the button `subtract background`.
- The message label in the status bar shows “select initial data point”.
- Click on the trace to select the start of the interval.
- The message label in the status bar shows “select final data point”.
- Click on the trace to select the end of the interval.
- The spectrum view shows the total spectrum in blue and the background subtracted spectrum in red; the title indicates the range, the message label in the status bar shows “no pending operation”.

Chapter 12

Step-by-step Procedures

12.1 Easy Edit of Blobs

12.1.1 Open data file and define ROI

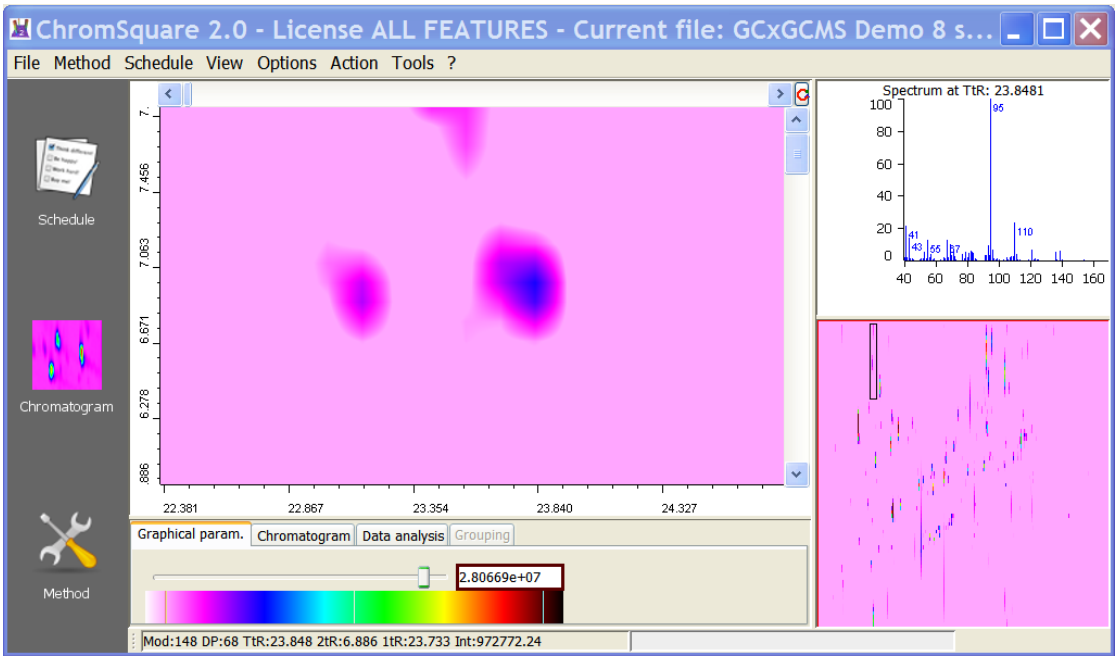


Figure 12.1: Zoom to the current ROI

1. Open the usual sample data file `Shimadzu GCxGC-MS 8sec.QGD`.

2. Switch to the Method Panel.

3. Fill in the following values of ROI: 22.3656, 24.7983.

4. Fill in the following values: Minimum Area 1,000,000, Noise 5000.

5. Switch to the Chromatogram Panel.

6. Move the mouse in the Map View and click the **right button** (just click, without dragging).

7. From the context menu, select `"Zoom to ROI"`.

8. The Map will show the region corresponding to the ROI (see Figure 12.1)

12.1.2 Integrate ROI and identify the Blobs

1. From the Action Menu, select `"Integrate ROI"` (you may also use the corresponding icon in the lower panel: switch to the Data Analysis tab and then click on the second icon). The results of the integration will be shown as straight lines contours which bound the two main blobs. See Figure 12.2. The Blobs are also reported in the Data Analysis table; in both cases, the Blobs are marked as "unknown", since identification has not yet been performed.

2. Draw a moving rectangle in the Map View, using the usual procedure (click the right button in the upper left corner of the rectangle; move the mouse while pressing the right button; release the mouse button in such a position that the rectangle will contain most part of the left Blob.

3. From the context menu, select `"Define Blob"` (see Figure 12.3)

4. A dialog box will open, asking for the name to be assigned to the selected Blob (12.4); answer "Blob A", and close the dialog with the "Ok" button.

5. Repeat the procedure for the right Blob; assign the name "Blob B".

6. Switch to the Method Panel: the Blob Table now will show the two Blobs defined by the user (see Figure 12.5). Check that the Total Retention Times values reported in the Blob Table match the values displayed in the Chromatogram Panel.

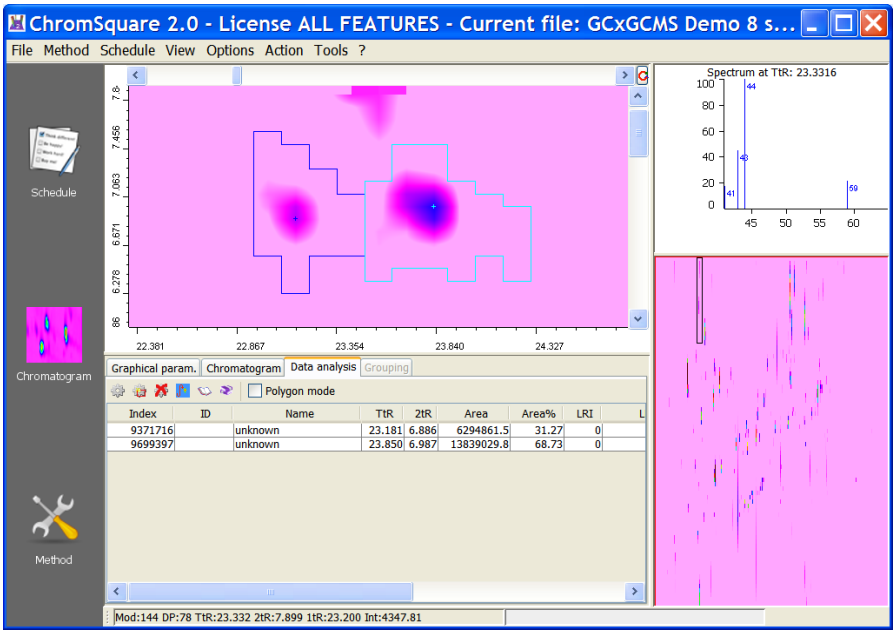


Figure 12.2: Integrate the current ROI

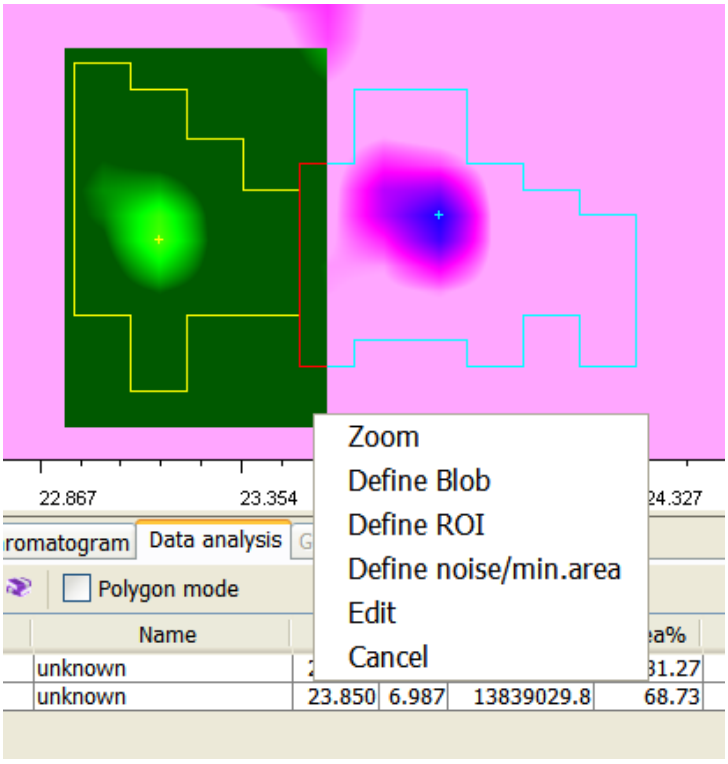


Figure 12.3: Define Blob

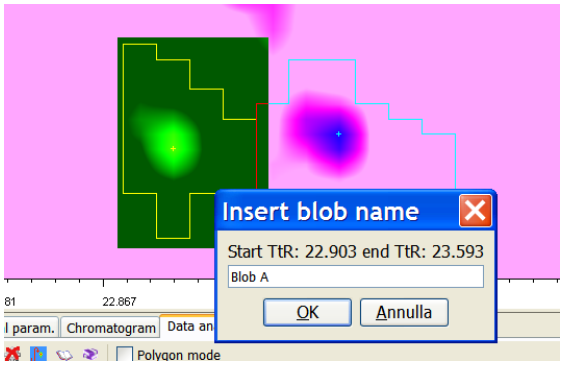


Figure 12.4: Insert Blob name

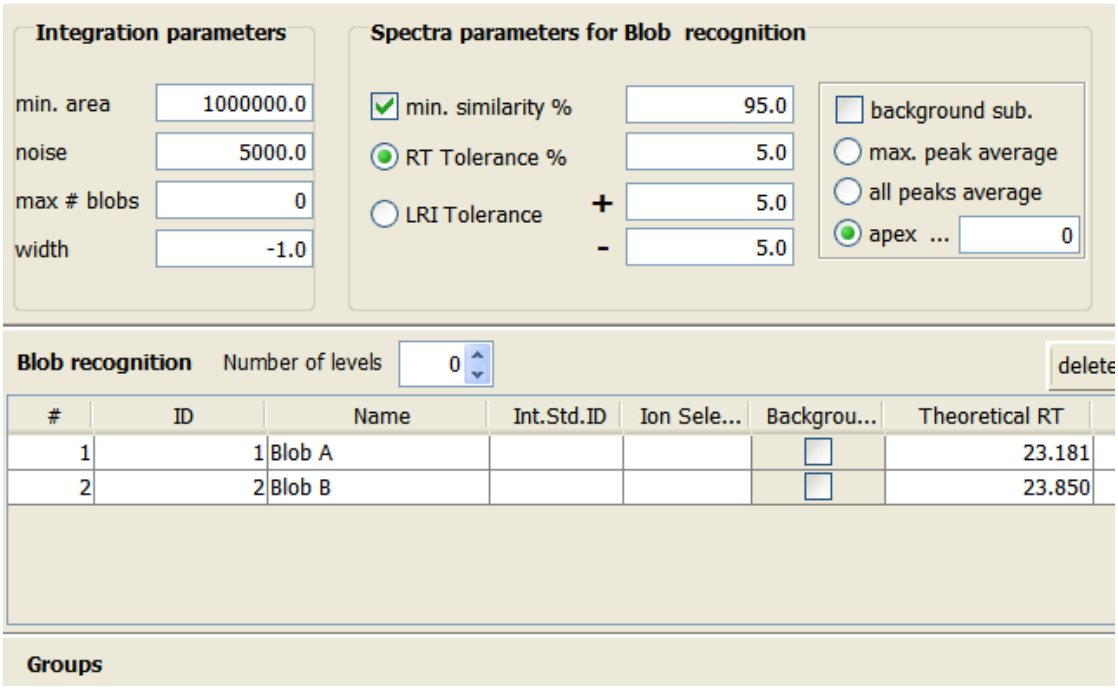


Figure 12.5: The Method Panel with the user defined Blobs

7. Switch to the Chromatogram Panel and execute again `Integrate ROI`: the Blobs will now be identified, with the respect to the ones defined in the Method: their names are shown both in the Map View and in the Data Analysis table (see Figure 12.6).

8. With the Menu `Method|Save Method`, assign a name to the current Method (for example, `editblob1`) and save it;

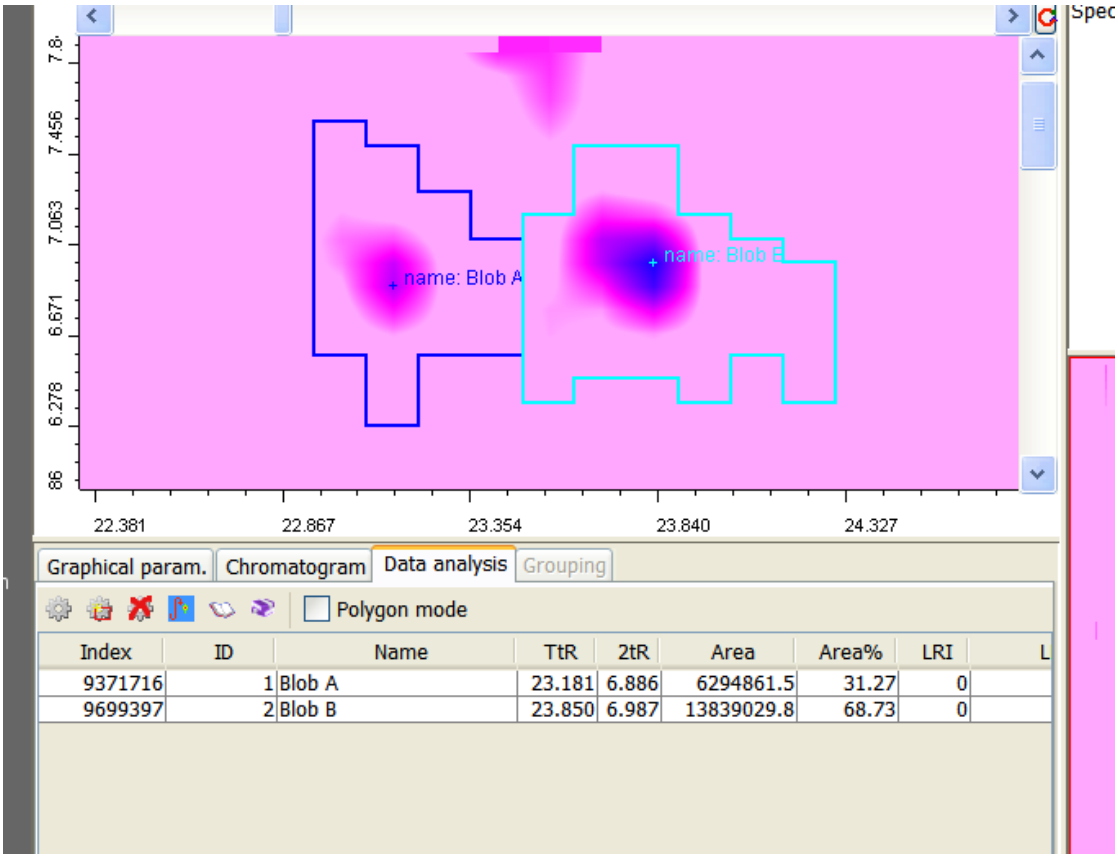


Figure 12.6: The Chromatogram Panel with the user defined Blobs

12.1.3 Define a Group

Let us define a Group which includes the two Blobs defined above.

1. Switch to the Method Panel.

2. In the Group Section, press the `Add group`; from the dialog box that will pop-up, select both Blobs (holding down the CTRL key) and give Ok.

3. The Method Panel will now display a new Group, formed by the two existing Blobs (see 12.7).

4. Switch to the Chromatogram Panel and Integrate the ROI again. Apparently nothing changes, except that the "Grouping" tab in the lower panel is now active. Click on it.

5. The Grouping panel will show the new Group and its area value, given by the sum of the areas of the two blobs (see 12.8).

Figure 12.7: The Group section of the Method Panel

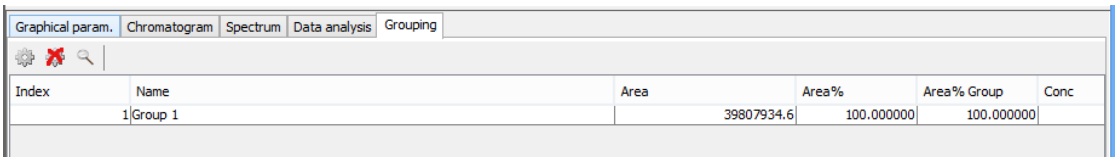


Figure 12.8: The Grouping tab of the Chromatogram Panel

12.1.4 Merge Blobs

With this operation the user can *add* a *Blob* (that we call the *origin* Blob) to another one (that we call the *destination* Blob); the result is a new Blob, which is the *union* (or *merging*) of the *origin* and *destination* Blobs. The new Blob will be added to the Blob list, while the original ones will be deleted.

A set of conditions should be satisfied, in order that the merging operation could be performed: the original Blobs must be *adjacent*, and the user should correctly select the two Blobs.

Starting from the sample of the previous paragraphs, suppose you want to *add the Blob B to the Blob A*.

The operation may be summarized as follows:

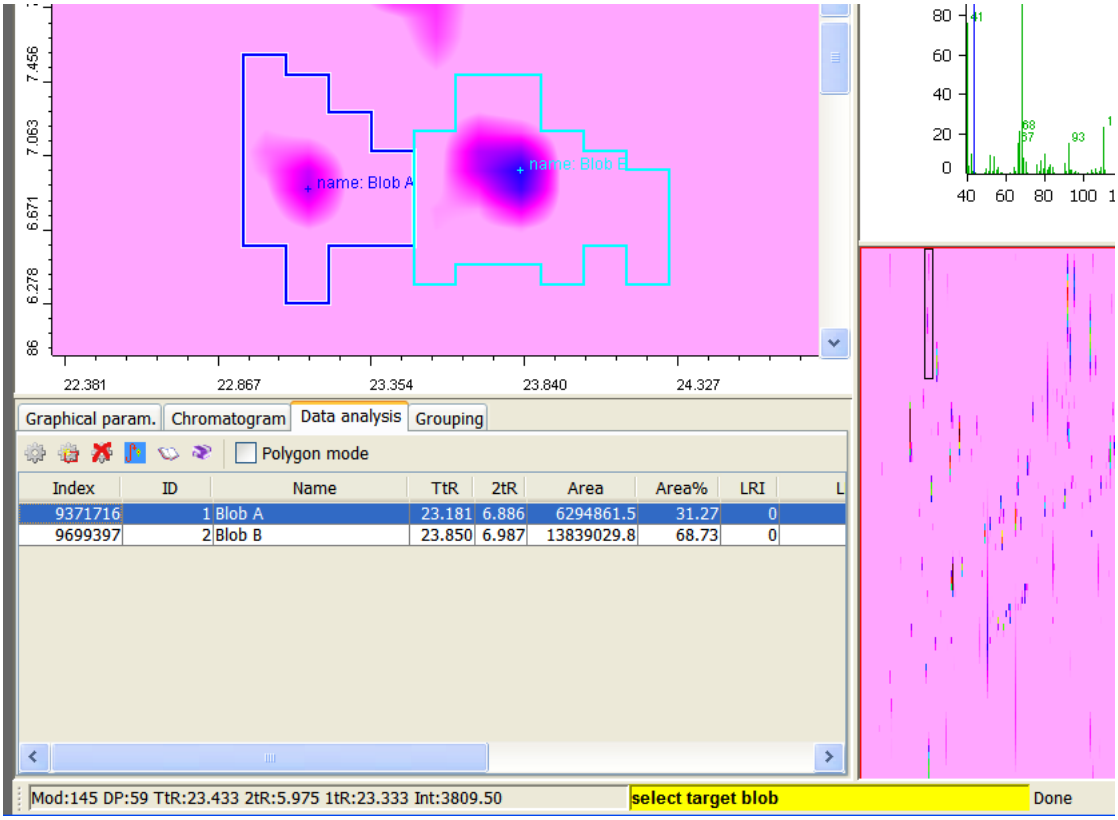


Figure 12.9: Merging Blobs: origin Blob selected - ask for target

1. Switch to the Chromatogram Panel. In the lower window, select the Data Analysis page.

2. Move the mouse in the Map View and position it at any point *inside* the Blob A.

3. Left click the mouse to select the Blob. Note that:

   - The currently selected Blob is emphasized with a *double border*.
   - The currently selected Blob is also selected (marked with a *blue* background) in the Blob list of Data Analysis.

4. Click the **right button** (just click, without dragging).

5. From the context menu, select `Add to Blob`: a warning message, with the text "`select target blob`" on a *yellow* background will be displayed in the Status bar. See Figure 12.9.

6. Move the mouse to the preferred target Blob and select it by left clicking inside it.

7. The result of the merging operation will be immediately displayed, both on the Map View and on the Data Analysis table, as shown in Figure 12.10.

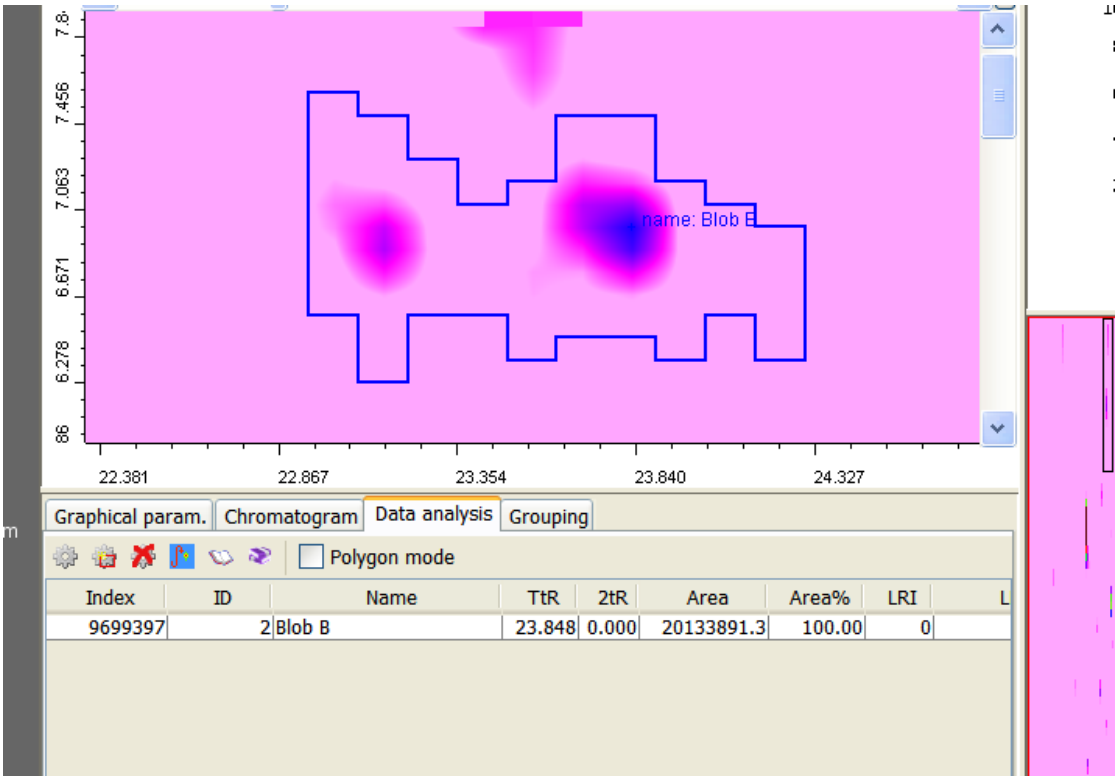


Figure 12.10: Merging Blobs: the results

8. Check that:

   - The Data Analysis table now contains just one Blob.
   - The Map View shows now the merged Blob, which gets the name of the *destination* (in fact, the operation is add A to B, which means that B maintains its identity).
   - The area of the merged Blob is the sum of the areas of the two original Blobs: this can also be verified by comparison with the area of the Group 1, which was obtained as the sum of the areas of the same original Blobs (see 12.1.3).

12.1.5 Add a Peak to a Blob

Adding a Peak to a Blob is very similar to the Merge Blobs described above (see section 12.1.4). Even the context menu choices and the other commands are the same, with the following difference:

- If the user initially clicks the mouse inside a Blob, then a "Merge Blobs" operation is started.
- If the user initially clicks the mouse neare a Blob, then a "Add a Peak to a Blob" operation is started.

The step-by-step procedure is here reported for the "Add Peak" case. The procedure is the continuation of the one described in previous section.

1. Switch to the Chromatogram Panel.
2. Move the mouse in the Map View and position it at a point *near* the Blob. More precisely, the point should belong to a modulation adjacent to the Blob; for example, since the existing Blob spans from modulation 141 to 151, choose a point of modulation 152.
3. Click the **right button** (just click, without dragging).
4. From the context menu, select `Add to Blob`: a warning message, with the text `"select target blob"` on a *yellow* background will be displayed in the Status bar. See Figure 12.9.
5. Move the mouse to the target Blob and select it by left clicking inside it.
6. The result of the merging operation will be immediately displayed, both on the Map View and on the Data Analysis table, as shown in Figure 12.11.
7. Check the difference between Figures 12.10 (before adding the peak) and 12.11 (after adding the peak): the Blob in the second Figure is slightly larger, since it includes a new Peak on the right side. Also the area is larger.

12.1.6 Removing Peaks from a Blob

One or more Peaks can be deleted from the left or right sides of a Blob.

The sample procedure is the continuation of the one described in previous section: it will be used to delete the Peak that had been just added to the Blob, restoring the situation at the end of section 12.1.4, Figure 12.10.

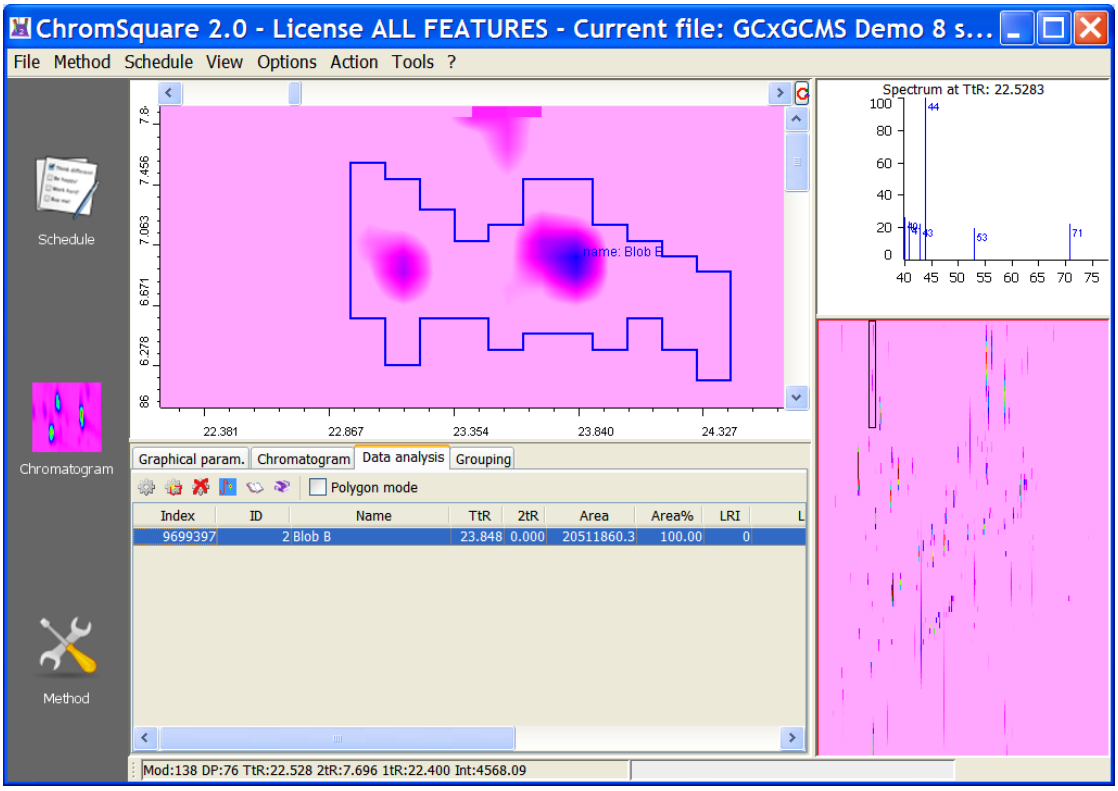


Figure 12.11: Adding a Peak to a Blob: results

1. Switch to the Chromatogram Panel.

2. Move the mouse in the Map View and position it at a point *inside* the Blob A, near the right side. More precisely, the point should belong to the modulation 152, that is the one corresponding to the Peak that had been added.

3. Click the **right button** (just click, without dragging).

4. From the context menu, select `"Remove from Blob"`.

5. The modified Blob is displayed, both in the Map View and in the Data Analysis table.

6. Verify that the Blob features are exactly the same shown in Figure 12.10. In other words, the operation of Removing the Peak has canceled the operation of Adding a Peak.

12.2 UV Spectra Handling

12.2.1 Open a sample data file

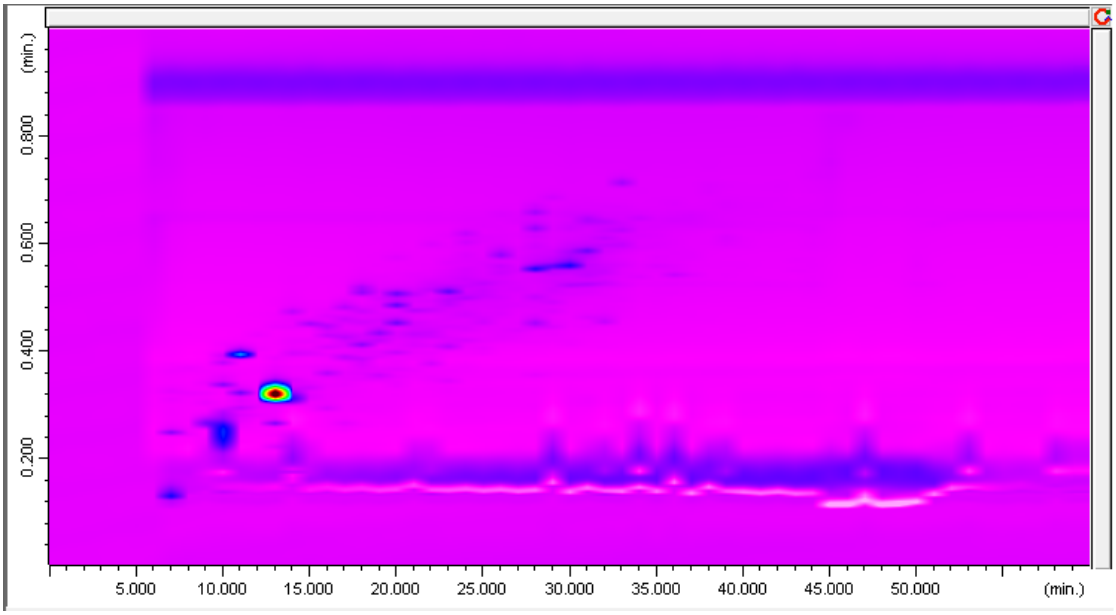


Figure 12.12: Sample datafile containing UV spectra

1. Select the menu command `File|Open Datafile`. A File Selection Dialog Box will open.
2. Select the sample data file "`LabSolutions Data Sample 1min.lcd`".
3. Check that the modulation time is 1 minute.
4. From the `Profile` combo box, select "`Shimadzu HPLC PDA Ch.1`".
5. The Map will show the datafile (see Figure 12.12)
6. In the Spectrum View, select the page "UV Spectrum".
7. Move the mouse in the Map View: the Spectrum View will show the UV spectrum of the point corresponding to the current mouse position. The spectrum is represented as a *black* line. The Figure 12.13 shows the UV spectrum at the top of the main Blob (Retention Time = 13.327).

12.2.2 Set a reference spectrum and compare with the current one

1. With the mouse pointer at a "reference point", right click to display the Map Plot Context Menu and select "`Set UV Spectrum as Reference`";

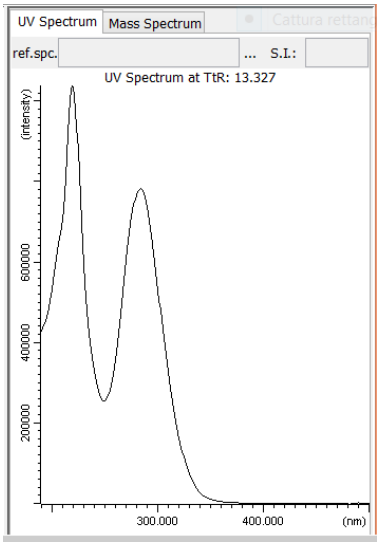


Figure 12.13: The UV spectrum at the top of the main Blob

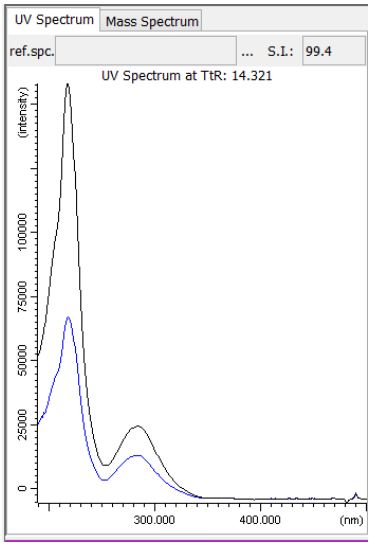


Figure 12.14: Current UV Spectrum vs. Reference Spectrum

2. The reference spectrum will be shown in the Spectrum View as a *blue* line, together with the black line of the current spectrum; while moving the mouse in the Map View, the blue line remains fixed, wheres the black one follows the mouse movements. The text field "`S.I.`" is constantly updated with the Similarity Index of the two spectra (see a sample in Figure 12.14)

12.2.3 Save a spectrum and then load it as reference

1. With the mouse pointer at a "reference point", right click to display the Map Plot Context Menu and select "`Set UV Spectrum as JCamp`". The JCamp file is generated (no message is displayed).

2. Using "My Computer" browse to the directory containing the datafile and check that a new file, having extension "`.jdx`", has been generated, and that the name corresponds to the retention time of the reference point.

3. Click on the "three dots" symbol on the toolbar of the Spectrum View; a File Selection Dialog Box will open, allowing to choose a JCamp file to be used as reference spectrum.

4. The reference spectrum will be shown in the Spectrum View as a *blue* line, together with the black line of the current spectrum, exactly like in the case described at 12.2.2.