

Chrom^{square} 1.5 User Manual

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

Chromaleont srl

September 2012

Contents

1	Foreword	14
1.1	Contact Information	14
1.2	Legal Information	14
1.2.1	Copyright	14
1.2.2	Warranty	15
1.3	Introduction	16
1.3.1	Outline of the Software	16
1.3.2	Outline of the Manual	17
2	Installation and Startup	19
2.1	Installing the software	19
2.2	Uninstalling the software	21
2.3	Starting ChromSquare software	22
2.4	Registering the License	23
2.4.1	License policy	23
2.4.2	License management	24
3	<i>Chrom^{square}</i> Overview	27
3.1	General Terminology and Concepts	27
3.1.1	Chromatogram	27
3.1.2	Chromatographic Map	28
3.1.3	Integration	28

3.1.4	Peaks	29
3.1.5	Blobs	29
3.1.6	Blob IDs	30
3.1.7	Blobs vs. Blob IDs	31
3.1.8	Methods	31
3.1.9	Units	32
3.2	<i>Chrom^{square}</i> Graphical User Interface	32
3.3	File Management	34
3.3.1	Raw Data Files vs. ChromSquare Files	34
3.3.2	<i>Chrom^{square}</i> Result Files	35
3.3.3	<i>Chrom^{square}</i> Schedule Files	36
3.3.4	<i>Chrom^{square}</i> Method Files	37
3.3.5	Opening Files	37
3.4	The Java Console	37
3.4.1	The Java Console buttons	38
4	Menu Bar	40
4.1	Menu File	40
4.1.1	Open Datafile	40
4.1.2	Close Datafile	43
4.1.3	Assemble Datafile	43
4.1.4	Save Datafile	43
4.1.5	Export as CDF file	44
4.1.6	Export as XML for Excel	44
4.1.7	Save Map as Image	44
4.1.8	Data info	46
4.1.9	Exit	46
4.2	Menu Method	46
4.2.1	New Method	47

4.2.2	Open Method	47
4.2.3	Save Method	47
4.2.4	Save Method As...	48
4.3	Menu Schedule	48
4.3.1	New	48
4.3.2	Add chromatograms	49
4.3.3	Open Schedule	49
4.3.4	Save Schedule As...	49
4.4	Menu View	49
4.4.1	Reset Zoom	50
4.4.2	Zoom In	50
4.4.3	Zoom Out	50
4.4.4	3D View	50
4.5	Menu Options	51
4.5.1	Interpolation switches	52
4.5.2	2D Chromatogram Plot switches	52
4.5.3	Linear Chromatogram Plot switches	54
4.6	Menu Action	54
4.6.1	Integrate ROI	55
4.6.2	Integrate Whole Chromatogram	55
4.6.3	Quantitate	56
4.6.4	Remove Integration	56
4.6.5	Integrate all chromatograms	56
4.6.6	Create Calibration	56
4.6.7	Show Calibration	57
4.6.8	Create Calibration Group	57
4.6.9	Show Calibration Group	57
4.6.10	Create Chromatogram Report	57
4.6.11	Search selected Blob	60

4.6.12	Search all Blobs	60
4.6.13	Correct Wrap Around	60
4.7	Menu Tools	60
4.7.1	Report Template Editor	60
4.7.2	Compare/Subtract Chromatograms	62
4.8	Menu Help	62
4.8.1	Help	62
4.8.2	License	62
4.8.3	Java 3D Info	63
4.8.4	Java Console	63
4.8.5	About	63
5	Schedule Panel	64
5.1	Schedule Tool Bar	64
5.2	Schedule Table	65
5.2.1	The Context Menu of the Schedule Table	67
6	Method Panel	71
6.1	Panel contents	71
6.1.1	Integration page	72
6.1.2	Method Filename	72
6.1.3	Method Parameters	72
6.1.4	Blobs ID Table (Blob Recognition)	72
6.1.5	Buttons for Blob ID Table management	73
6.1.6	Groups ID Table	74
6.1.7	Buttons for Group Table management	74
6.1.8	ROI	75
6.1.9	Ion Selection for Map Display	75
6.2	Shimadzu Spectra Search page	75
6.2.1	Library file #n	75

6.2.2	Delete duplicates	76
6.2.3	Reverse search	76
6.2.4	LRI upper tolerance-lower tolerance	76
6.2.5	LRI index in library	76
6.2.6	LRI Table	77
7	Chromatogram Panel	78
7.1	Panel Contents	78
7.2	The Map Plot Window	79
7.2.1	Features	79
7.2.2	Selection	79
7.2.3	Zoom	80
7.2.4	Map Plot Context Menu	83
7.3	The Graphical Parameters Page	86
7.3.1	The Color Mapping Panel	86
7.3.2	The Navigator Panel	88
7.4	The Chromatogram page	89
7.4.1	Single modulation in zoom interval	90
7.4.2	Single modulation in whole modulation interval	90
7.4.3	Multiple modulation in zoom interval	91
7.4.4	Multiple modulation in whole modulation interval	92
7.5	The Spectrum page	93
7.6	The Data Analysis page	93
7.6.1	The Data Analysis table	94
7.6.2	The Data Analysis Toolbar	99
7.6.3	The Data Analysis Context Menu	100
7.7	The Grouping page	106
7.8	The Status Bar	107
8	Qualitative Data Analysis	108

8.1	Set up the Chromatogram	108
8.1.1	Load the Chromatogram	108
8.1.2	View the Chromatogram	108
8.2	Integrate the Chromatogram	109
8.2.1	Set up the Method	110
8.2.2	Integrate Whole Chromatogram	111
8.2.3	Integrate ROI	111
8.2.4	Check the Integration Results	112
8.2.5	Change the Integration Parameters	112
8.3	Identify the Blobs	114
8.3.1	Manual Identification of the Blobs	114
8.3.2	Automatic Identification of the Blobs	115
9	Quantitative Data Analysis	118
9.1	External Standard Calibration	118
9.1.1	Set up the Schedule	118
9.1.2	Set up the Method	123
9.1.3	Load a chromatogram	126
9.1.4	Integrate the chromatogram	127
9.1.5	Identify the Blobs	129
9.1.6	Integrate again	130
9.1.7	Integrate All Chromatograms	131
9.1.8	Assign the concentrations	132
9.1.9	Save the Schedule	134
9.1.10	Create the Calibration	134
9.1.11	View Calibration results	136
9.1.12	Save the Schedule again	136
9.2	Internal Standard Calibration	138
9.2.1	Set up the Schedule	138

9.2.2	Set up the Method	138
9.2.3	Load a chromatogram	138
9.2.4	Integrate the chromatogram	138
9.2.5	Identify the Blobs	138
9.2.6	Integrate again	139
9.2.7	Integrate All Chromatograms	139
9.2.8	Assign the concentrations	139
9.2.9	Save the Schedule	139
9.2.10	Create the Calibration	139
9.2.11	View Calibration results	139
9.2.12	Save the Schedule again	140
9.3	Quantitation of an Unknown Sample Chromatogram	140
9.3.1	Add unknown sample files to the schedule	140
9.3.2	Load a chromatogram	140
9.3.3	Integrate the chromatogram	140
9.3.4	Execute the Quantitative Analysis	141
9.4	Group Calibration	142
9.4.1	Description	142
9.4.2	Define Groups in the Method Panel	143
9.4.3	Integrate All Chromatograms	147
9.4.4	Group Calibration	149
9.4.5	Group Quantitation	154
10	Advanced Topics	156
10.1	3D View	156
10.1.1	Description	156
10.1.2	Changing the scene	158
10.1.3	The 3D View Tool Bar	158
10.2	Compare/subtract tool	161

10.2.1	Introduction	161
10.2.2	Definitions	161
10.2.3	Description of the Compare/Subtract window	162
10.2.4	Operations	165
10.3	Define Polygonal Blobs	165
10.4	Profiles	168
10.4.1	Description	168
10.4.2	Defining a new Profile	168
10.5	Configuration	170
10.5.1	Description	170
10.5.2	The file <code>Options.xml</code>	170
10.6	Ion selection	171
10.6.1	Description	171
10.7	Assembling Data Files	172
10.7.1	Description	172
10.7.2	Procedure	173
11	Technical Notes	177
11.1	Prerequisites to install ChromSquare with GCMSsolution spectra search	177
11.1.1	Description	177

List of Figures

2.1	The Chromsquare desktop icon	22
2.2	The Chromsquare initial <i>splash screen</i>	23
2.3	Enter License Code window for Normal License	25
2.4	Enter License Code window for Demo License	26
3.1	<i>Chrom^{square}</i> Main Window	33
3.2	The Java Console	39
4.1	The Menu File	40
4.2	File description and features of the Data file selected	42
4.3	The Save Map as Image Dialog Box	45
4.4	The Select Font Dialog Box	45
4.5	The Current Data Info Dialog Box	46
4.6	The Menu Method	47
4.7	The Menu Schedule	48
4.8	The Menu View	50
4.9	The Menu Options	51
4.10	The Menu Action	55
4.11	The Report Template Selector Dialog Box	58
4.12	The Chromatogram Report Preview	59
4.13	The Menu Tools	61
4.14	The Report Template Editor Window	61
4.15	The Menu Help	62

4.16	The The Java 3D information window	63
5.1	The Tool Bar of the Schedule Panel	64
5.2	The Schedule Panel before loading a Method	68
5.3	The Schedule Panel after loading a Method	68
5.4	The Schedule Panel after the assignment of the Concentrations . . .	69
5.5	The context menu of the Schedule Table	70
6.1	The Method Panel - Integration Page	71
6.2	The Method Panel - Shimadzu Spectra Search Page	76
7.1	Map Plot - Context Menu	81
7.2	Map Plot - Zoomed Region	82
7.3	Insert Blob Name window	84
7.4	The Method Panel with the new Blob ID	84
7.5	Graphical Parameters Page	86
7.6	Navigator Panel with Zoomed Area	88
7.7	The Toolbar of the Chromatogram page	90
7.8	Single modulation in zoom interval	91
7.9	Single modulation in whole modulation interval	91
7.10	Multiple modulation in zoom interval	92
7.11	Multiple modulation in whole modulation interval	92
7.12	The Spectrum page	93
7.13	Data Analysis page - Overall view	94
7.14	Data Analysis page - Left view	94
7.15	Data Analysis page - Right view	95
7.16	Data Analysis page - Example showing ID Grouping case	98
7.17	Data Analysis page - The Data Analysis Toolbar	99
7.18	Enhanced representation of the selected Blob	100
7.19	Data Analysis page - The Data Analysis Context Menu	101

7.20	Show Spectrum window	102
7.21	A full zoomed Blob	103
7.22	The window displaying Blob Info	104
7.23	The Assign Compound dialog box	105
7.24	Method Panel showing one Group	106
7.25	Grouping page - The results	106
7.26	Chromatogram Panel - The Status Bar	107
8.1	The Method parameters	110
8.2	Qualitative Analysis: Whole Chromatogram integrated	111
8.3	Qualitative Analysis: Define ROI	113
8.4	Qualitative Analysis: ROI integrated	113
9.1	A list of files properly ordered for quantitative analysis	120
9.2	Initial Window for Quantitative Analysis	121
9.3	Schedule Panel with some Chromatograms added	121
9.4	The Schedule Menu with “Add chromatograms” command selected .	122
9.5	Switch to the Method Panel	123
9.6	The Method Menu with “Open Method” command selected	124
9.7	The Method Panel showing a new empty Method	125
9.8	The Method Panel with some parameters entered	125
9.9	The icon for loading the selected chromatogram	126
9.10	The Menu Action - Integrate Whole Chromatogram	127
9.11	The window showing the calculation progress	128
9.12	Integrated chromatogram - Zoomed view	128
9.13	Manual identification of a Blob	129
9.14	Method Panel showing Blob names and Method file name	130
9.15	Integrated chromatogram with names of the recognized Blobs . . .	131
9.16	Schedule panel ready to insert concentration values	133
9.17	Schedule panel while inserting a concentration value	134

9.18	The Calibration Results Dialog box	135
9.19	The Calibration Graph window	137
9.20	The Quantitation Results reported on the Schedule Panel	142
9.21	Method Panel with some Blob ID defined	144
9.22	List of all Blob IDs	145
9.23	Selection of the Blob IDs	145
9.24	Method Panel with one Group	146
9.25	Method Panel with more Groups	147
9.26	Integration Results with Named Groups	148
9.27	Integration Results: Grouping Results Table	149
9.28	Group Calibration Command	150
9.29	Group Calibration Summary	151
9.30	Group Calibration Data	152
9.31	Group Calibration Graph	153
9.32	Group Calibration - Quantitation Results - Data Analysis Page . .	154
9.33	Group Calibration – Quantitation Results – Grouping Page	155
10.1	3D View Window - Initial View	157
10.2	3D View Window - Surface colorization	160
10.3	Compare/Subtract Tool	162
10.4	The Compare/Subtract Window Tool Bar	163
10.5	Defining a Polygonal Region	166
10.6	Entering the polygon region name	167
10.7	The polygonal Blob after integration	167
10.8	Open Dialog Box with Profile Combo Box	169
10.9	The New Profile window	170
10.10	Method Panel with Ion Selection	172
10.11	The File selection Dialog box of the Assemble case	173
10.12	List of the files to assemble	174

10.13Files selected for assembling	174
10.14Asking for confirmation before assembling	175
10.15The resulting assembled chromatogram	176

Chapter 1

Foreword

1.1 Contact Information

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1.2 Legal Information

1.2.1 Copyright

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Information in this publication is subjected to change without notice and does not represent a commitment on the part of the vendor.

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.

1.2.2.3 Exceptions

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

1. Improper product handling.
2. Repairs or modifications performed by parties other than Chromaleont or Chromaleont designated companies.
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.
6. Turning OFF the product without following the proper shutdown procedure leading to device failures and damage to data and software, including the product's basic software.

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 re-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 LCsolution
3. LCxLC version for Shimadzu LCMSSolution and LCsolution
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 GCsolutionlicense 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 1-3;
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 from 4 to 7;
3. the third part is a detailed description of the main *routine operations*: namely, Qualitative and Quantitative Analysis; it includes Chapters 8 and 9;

4. the fourth part is a description of various *advanced operations*; it includes Chapter 10.

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 too many factors (like license types and choice of sample data files). In some 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.

Chapter 2

Installation and Startup

2.1 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 it¹.

Before installing the *Chrom^{square}* software verify that a recent *Java Runtime Environment* (version 1.6 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.

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

2. The next window is a *Welcome* window; no answer is required; select **Next** to continue.
3. The next window informs the user about the installed Java Environment; no answer is required; select **Next** to continue.
4. The next window informs the user about the installed Java Environment; select **Next** to continue.
5. The next windows is *Select Destination Location*. The software suggests as default the folder ChromSquare in the C: root disk. It is recommended not to change the default folder. Select **Next** to continue.
6. 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.
7. 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.

8. 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.
9. The *Installing* window is then displayed, showing a progress bar which lasts for some instants; at the end the window will close automatically.
10. The installation procedure terminates asking the user for some optional installations.
 - The first one 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 second 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 ChromSquare Setup Wizard*) terminates the installation procedure. If you want to launch immediately the program, tick the appropriate check box and then the **Finish** button.

2.2 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.

2.3 Starting ChromSquare software

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

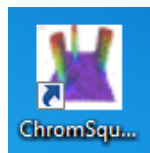


Figure 2.1: The Chromsquare desktop icon

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

The splash screen is shown in the Figure 2.2.



Figure 2.2: The Chromsquare initial *splash screen*

2.4 Registering the License

2.4.1 License policy

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

- *regular* licenses are based on an *hardware device* (Bluetooth Adapter key, or simply “BT” key);
- *demo* licenses are based on an *expiration date*.

In both cases:

- any license is supplied together with an *activation code*, that is a sequence of 32 *hexadecimal digits*².
- licenses (both regular and demo ones) are provided in different *license types*. Types are relative to a particular set of instruments. See the Section 1.3.1.1 for a list of the available types.

The first time that the software is executed, it asks the user to enter the *activation code*; in the case of demo license, it also asks for the *expiration date*.

²Hexadecimal digits are the 10 digits from 0 to 9 and the 6 characters from A to F.

If the data entered are correct, they are saved internally (so that they are non longer required the next time the software is executed) and the software session can start.

If they are not correct, the software will be terminated.

2.4.2 License management

When the software starts, it performs the following operations:

1. Before all, it checks if a BT key is present:
 - if it is present, the software assumes that this is a case of a *regular* license;
 - if it is not present, the software assumes that this is a case of a *demo* license.
2. A window is then displayed, showing the *Software License Agreement* and some fields to be compiled by the user:
 - The Activation Code, that the user had received together with the software CD; it may be entered manually (beware of entering exactly 32 hexadecimal digits), or may be copied and pasted if the license had been provided though an electronic medium.
 - The License Type, to be chosen through the Combo Box.
 - In the case of demo license, also the Expiration Date (in the format YYYY.MM.DD) should be entered.

Figure 2.3 shows the Software License Agreement window in the *Normal License* case.

Figure 2.4 shows the Software License Agreement window in the *Demo License* case.

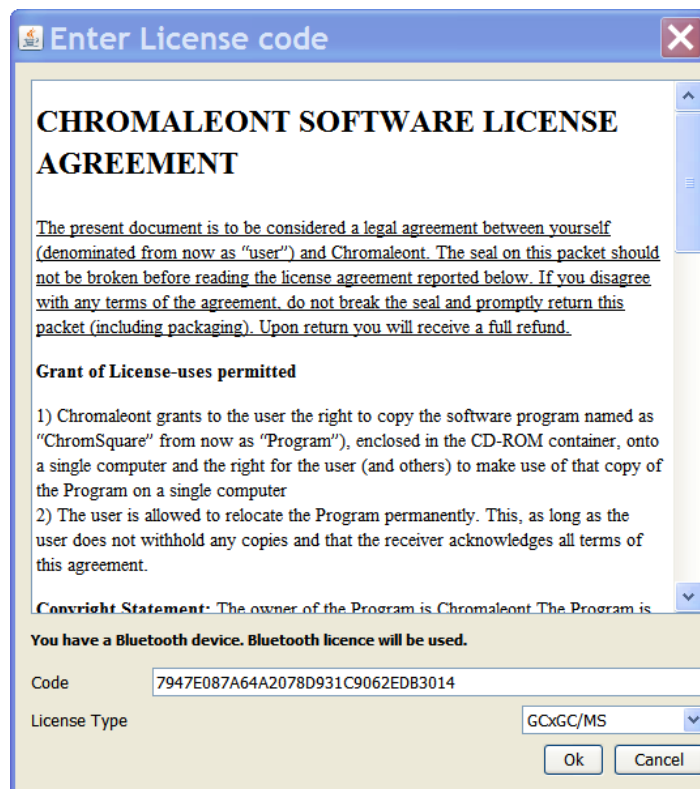


Figure 2.3: Enter License Code window for Normal License

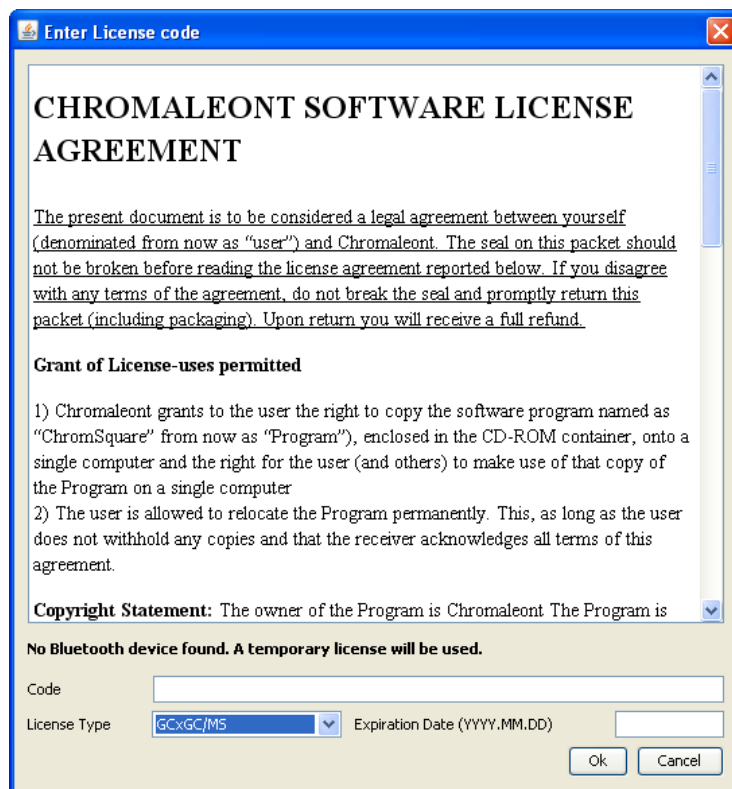


Figure 2.4: Enter License Code window for Demo License

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 Blob 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 area of the corresponding polygon.

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 7.6.1 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.

Blob ID is a data structure containing 3 elements:

- the ***Name***, that is name of the chemical compound(s) corresponding to the region; note that names may be very long character string;
- the ***ID***, that is an integer number, a sort of abbreviation used for easier reference when more chromatograms are compared among them (like in the calibration process);
- the ***Region***, that is the geometrical description of the polygonal area.

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

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 ChromSquare *Result File* (xxx).
- 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 xxx for details);
- a list of *Blob identifiers* (called also *Blob IDs*, see 3.1.6);
- an optional *Region of Interest* (also called ROI);

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

3.1.9 Units

Time is always represented internally in minutes; tables and graphs also show time in minutes, except in the particular cases, where time is represented in seconds.

Absorbance is normally represented in *microVolts*, except in the particular cases, where absorbance is represented in *milliVolts*. Anyway please refer of the manual of the software that generated the data file that has been imported, since ChromSquare 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 *ChromSquare Side Bar*, placed in the left side of the main window. This bar contains some large icons (presently 3, 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**.

In other words, the contents of the main window varies 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.

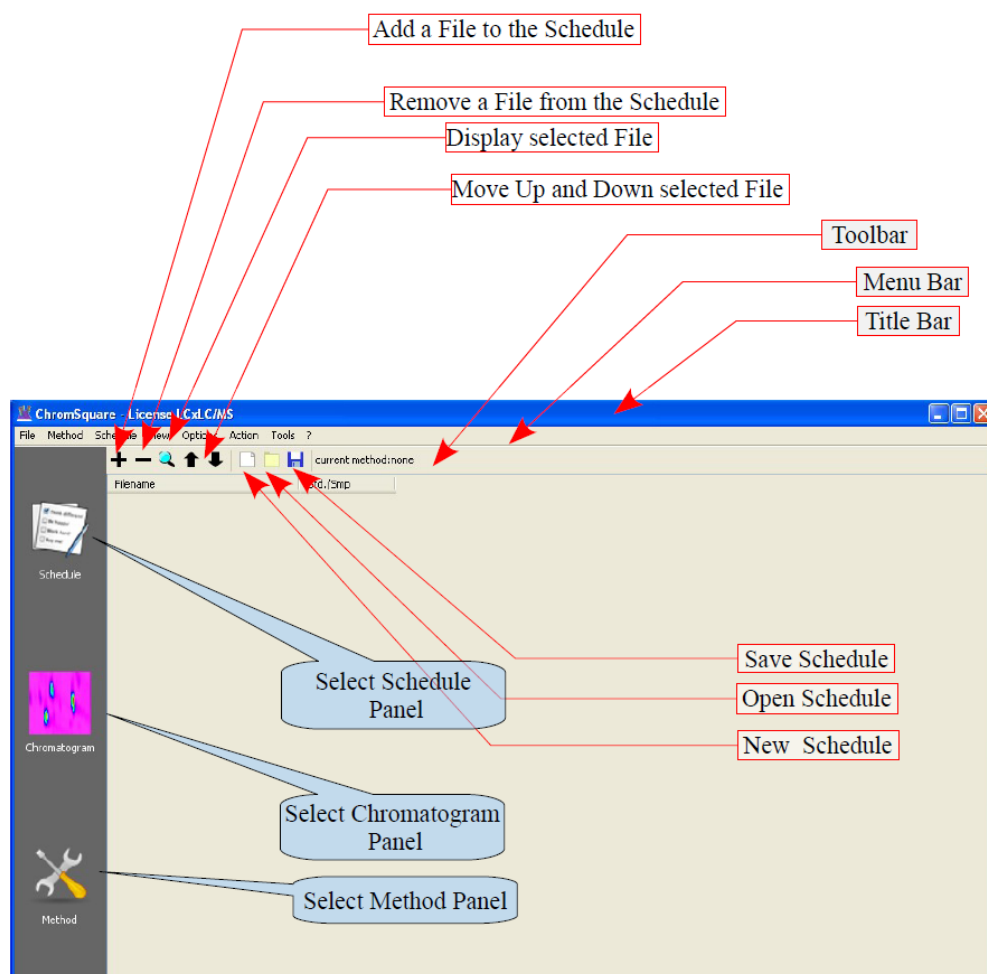


Figure 3.1: *Chrom^{square}* Main Window

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 Panel	This window is used to display the Schedule Panel for quantitative analysis.
Chromatogram Panel	This window 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 window is used to display the Method Panel , which lets to assign the method parameters.

3.3 File Management

3.3.1 Raw Data Files vs. ChromSquare 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 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). 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 xxx); initially it contains only the link to the Raw Data File and few input information (Modulation Period, Second Elution 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 xxx), 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 **Integrate All Chromatograms**, see xxx.
- **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 ChromSquare 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 window 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.

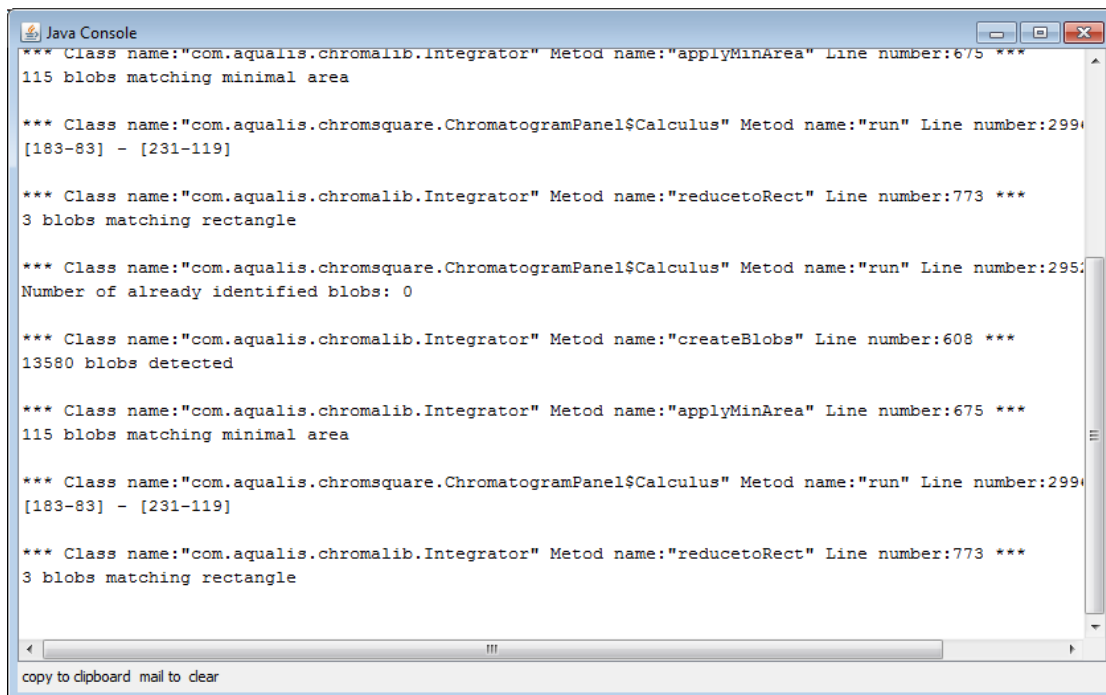


Figure 3.2: The Java Console

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

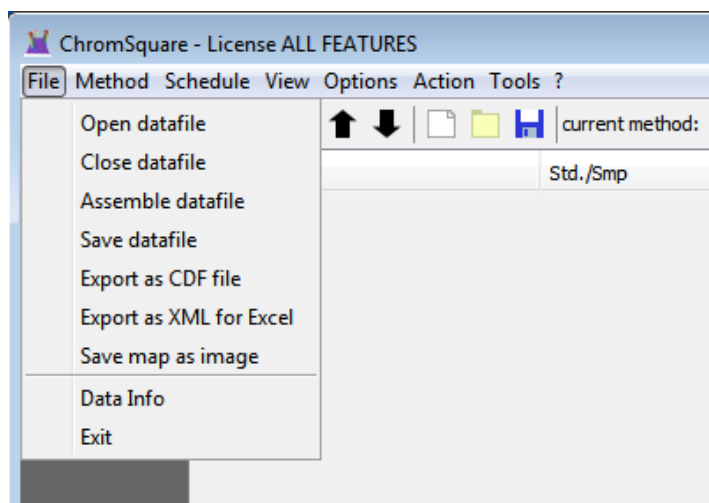


Figure 4.1: The Menu File

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”
- ChromSquare 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:

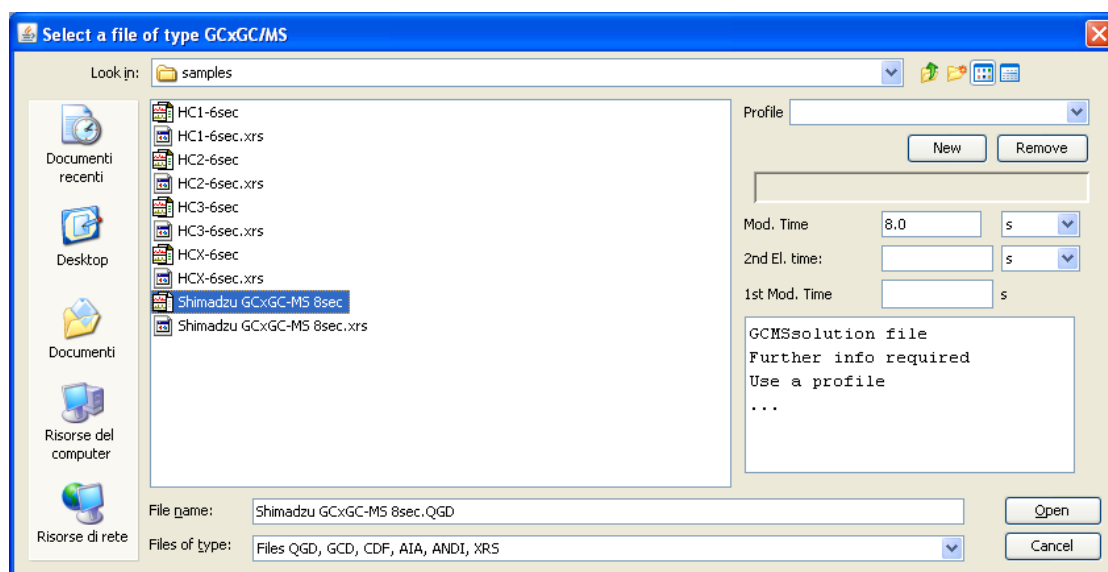


Figure 4.2: File description and features of the Data file selected

- the *upper part* is used to define and select a *profile* (see Section 10.4); since profiles are defined only for some kinds of files, this part is not always displayed;
- 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, 2nd elution time, 1st 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 10.7 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” extension (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).

4.1.6 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 4.3) also contains some customization controls:

¹NetCDF (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.

²AIA-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.

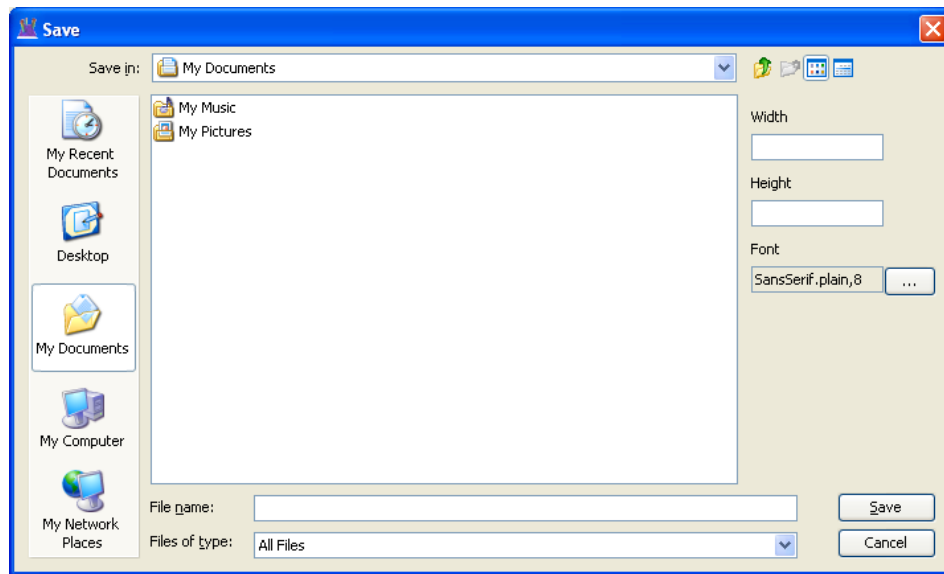


Figure 4.3: The Save Map as Image Dialog Box

- 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 4.4).

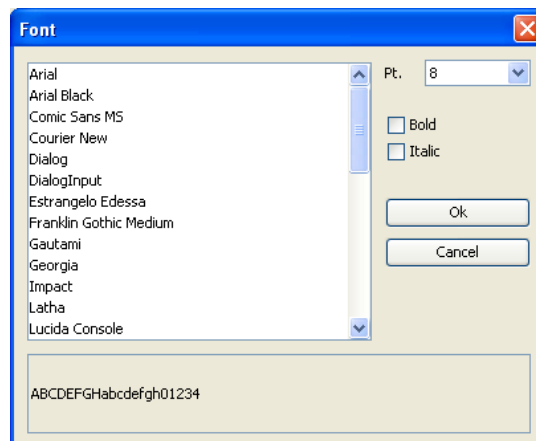


Figure 4.4: The Select Font Dialog Box

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 4.5.

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

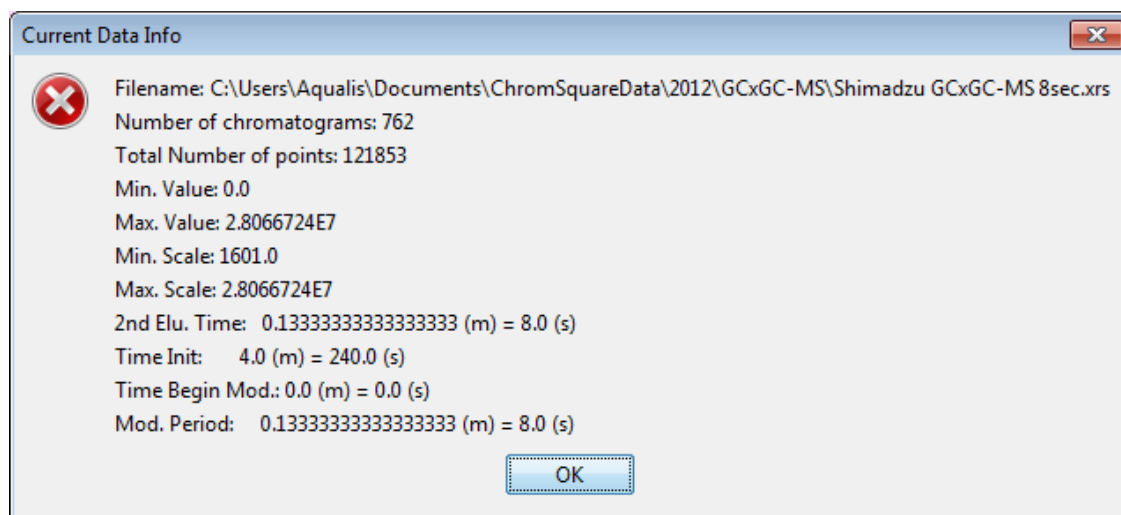


Figure 4.5: The Current Data Info Dialog Box

4.1.9 Exit

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

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 4.6.

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).

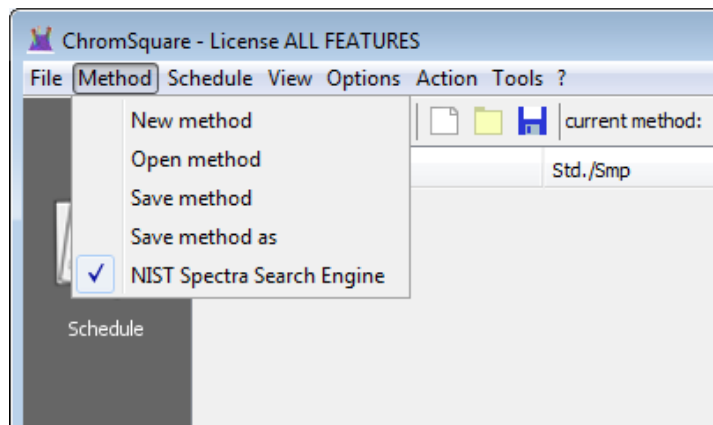


Figure 4.6: The Menu Method

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 6.

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 4.7

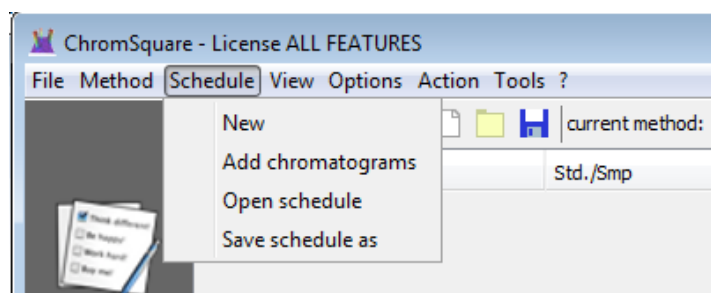


Figure 4.7: The Menu Schedule

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 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.

4.4 Menu View

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

The menu is shown in 4.8

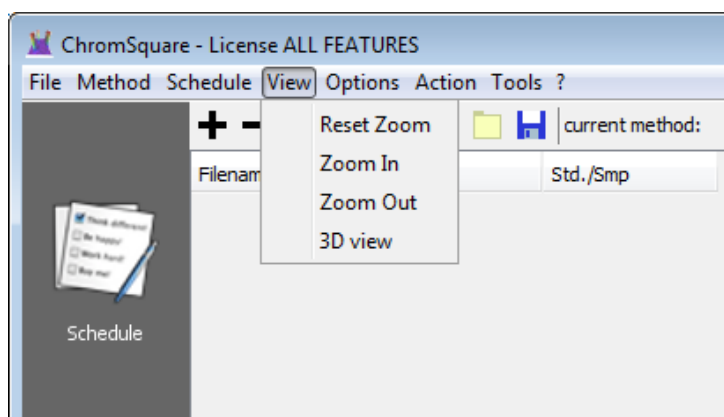


Figure 4.8: The Menu View

4.4.1 Reset Zoom

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

See Section 7.2.3 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 7.2.3 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 7.2.3 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 10.1 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 4.9.

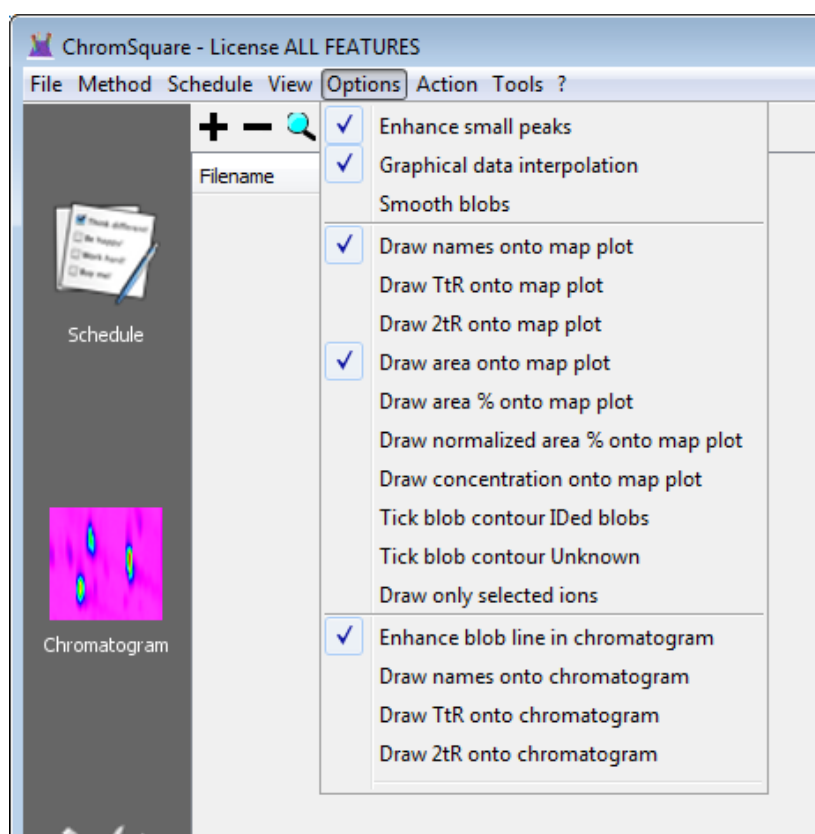


Figure 4.9: The Menu Options

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)

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 Graphical Data Interpolation

The switch enables the 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, generating an effect of a continuous shade of colors; if **OFF**, only the values of actual chromatographic data points are taken into account, generating an effect of a “pixelated” map.

4.5.1.3 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 6.1.9).

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.

4.6 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 4.10

Note: Figure 4.10 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.

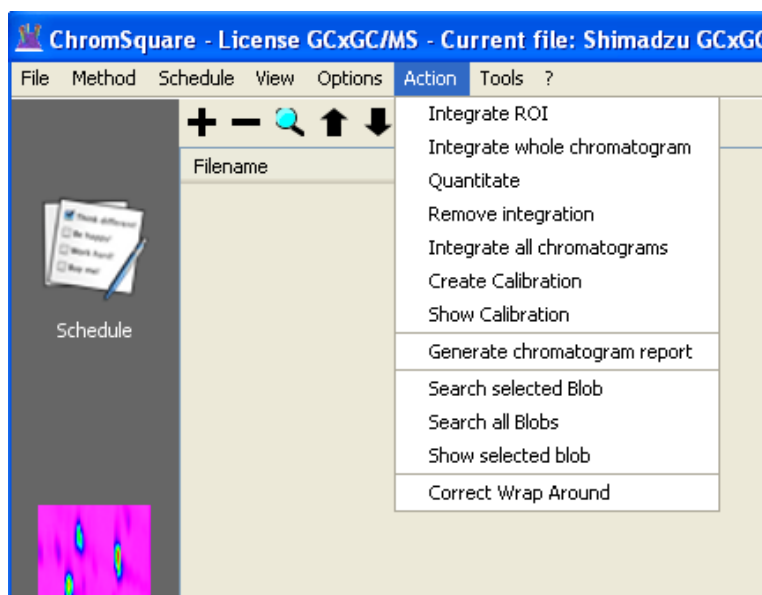


Figure 4.10: The Menu Action

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 6.1.8) and can be graphically defined through the mouse and context menu command (see Section 7.2.4.3).

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 4.9).
- 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 command 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

The command starts the procedure for the generation of a new *Calibration* curve, based on the current schedule.

See Section 9.1.10 for a detailed description of the procedure.

4.6.7 Show Calibration

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

4.6.8 Create Calibration Group

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

See Section 9.4.4 for a detailed description of the procedure.

4.6.9 Show Calibration Group

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

4.6.10 Create 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 4.7.1).

As first action, the Create Chromatogram Report command displays a pop up window, showing the list of available Report Templates (see Figure 4.11).

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 4.12

³The 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/>.

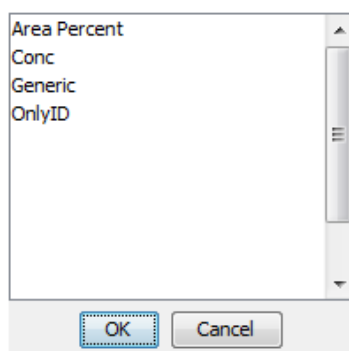


Figure 4.11: The Report Template Selector Dialog Box

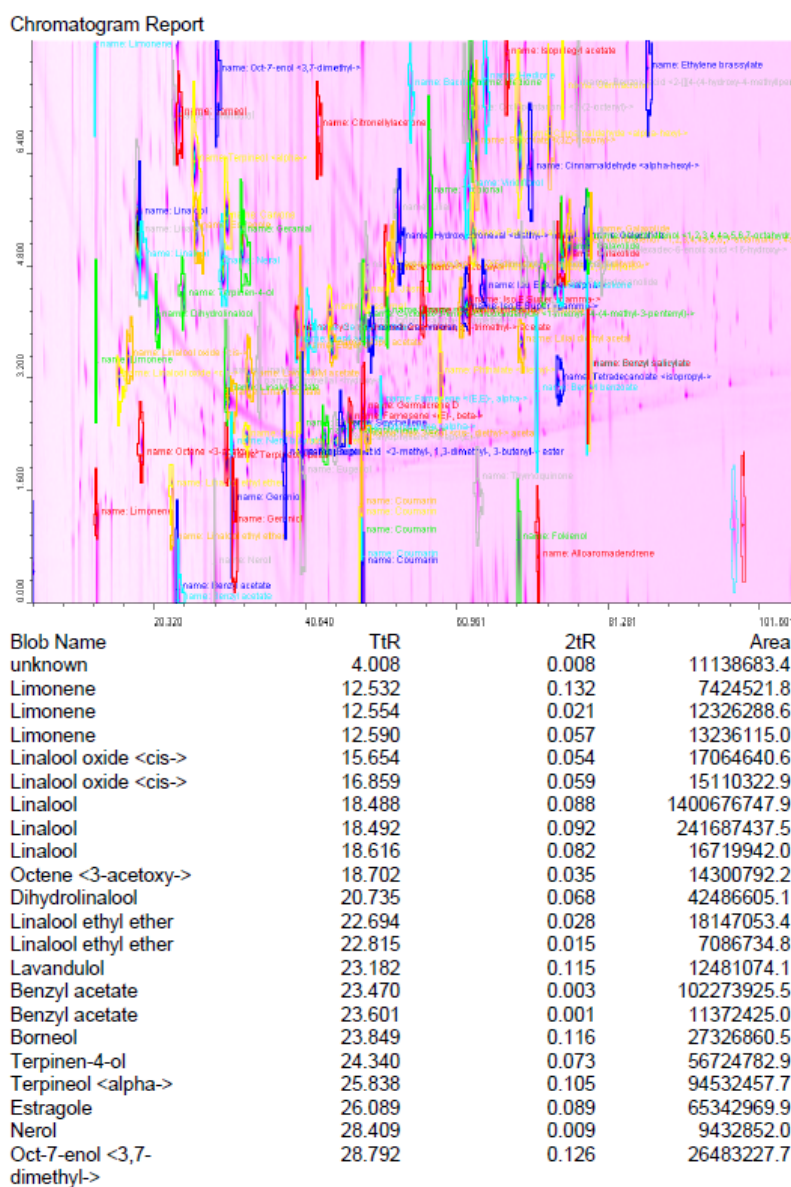


Figure 4.12: The Chromatogram Report Preview

4.6.11 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 7.6.3, to select the Blob).

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

4.6.12 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.

4.6.13 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 4.13

4.7.1 Report Template Editor

This command calls a special tool, the *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.

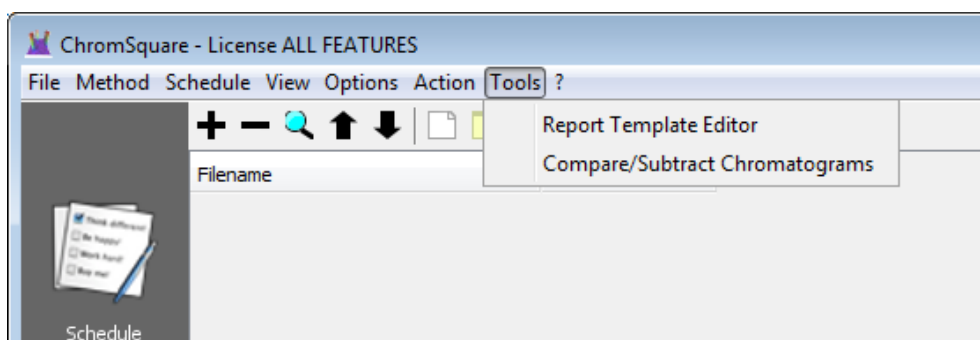


Figure 4.13: The Menu Tools

The Report Template Editor window is shown in 4.14

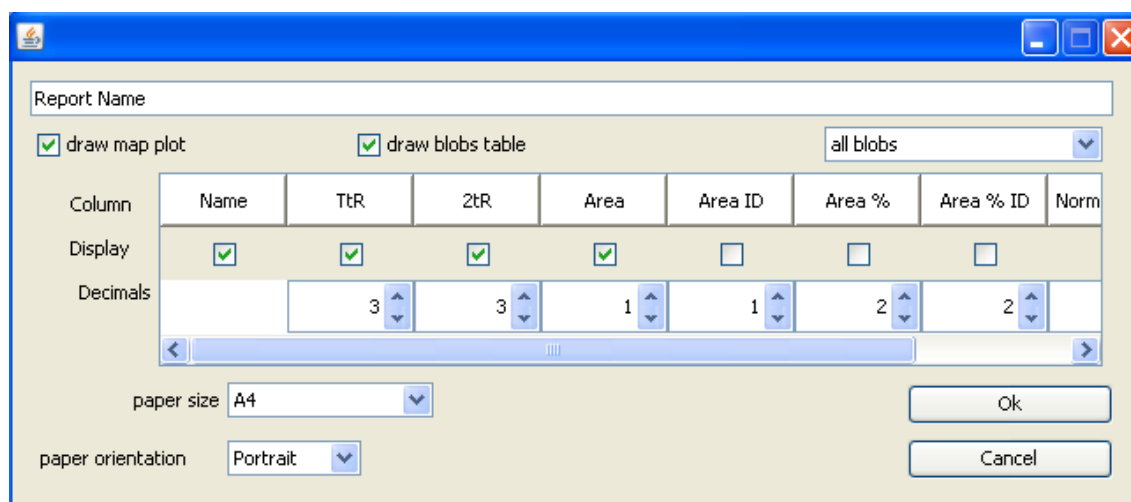


Figure 4.14: The Report Template Editor Window

The user can choose:

- paper format and orientation;
- whether the report should include the Plot Map or not;
- whether the report should include the Result Table or not;
- which columns of the Result Table have to be printed and, in the case of numerical values, how many decimal digits are requested.

The report name must be unique: if accidentally an existing name is chosen, the text appears in red. Once finished, pressing the “Ok” button, the report template is automatically generated and saved.

4.7.2 Compare/Subtract Chromatograms

The command opens a special window designed to perform the comparison and subtraction of chromatograms.

The tool is described in detail in Section 10.2.

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 4.15

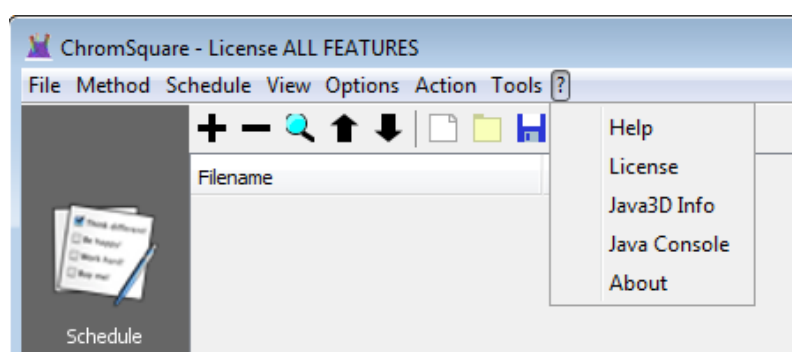


Figure 4.15: The Menu Help

4.8.1 Help

The command shows the ChromSquare User Manual in an Acrobat Reader window.

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 10.1) 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 4.16.

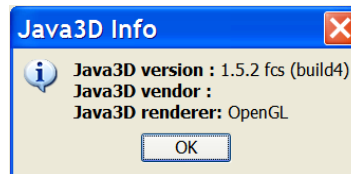


Figure 4.16: The The Java 3D information window

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 ChromSquare 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*.

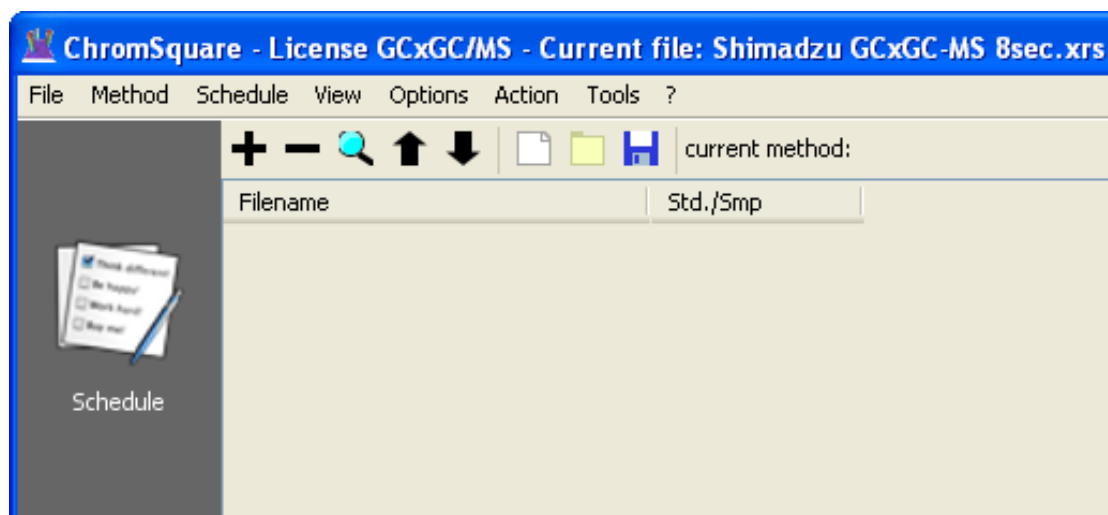


Figure 5.1: The Tool Bar of the Schedule Panel

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.

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. **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.
3. **Magnifying Lens:** Loads the selected chromatogram from the Raw Data file.
4. **Arrow Up:** Moves the selected chromatogram one row up.
5. **Arrow Down:** Moves the selected chromatogram one row down.
6. **“New” symbol:** Creates a new empty Schedule. Corresponds to the menu command 4.3.1.
7. **“Open” symbol:** Opens an existing Schedule. Corresponds to the menu command 4.3.3.
8. **Floppy Disk symbol:** Saves the current Schedule. Corresponds to the menu command 4.3.4.

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* 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. As long as chromatograms are added to the schedule list, new rows are added to the table, reporting the chromatogram filename in the first column of the table, leaving for the moment empty the Standard-Sample option of the second column; see Figure 5.2.
3. When the user defines a Method (this may be accomplished in different ways, see the Method Panel chapter for details), the table is filled with new columns, *each column corresponding to a Blob ID* defined in the Method. The new columns have headers that report the names of the Blob IDs. The columns are designed to contain the *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. Initially all concentrations are set equal to zero.
4. At this point, the user can fill in the table: this is a two-step procedure. *The first step is the choice between Standard or Sample*: this is easily done using the combo boxes of the second column. *The second step is the assignment of the concentrations to the Blob IDs of the chromatogram that should be used as standards for the calibration*. The user should assign the concentrations, filling the table cells, according to the following criteria:
 - Chromatograms defined as *standards* have all concentrations different from zero;

- Chromatograms to be considered as *unknown samples maintain zero concentrations for all Blobs, with the exception of the Blobs that are referenced as internal standards, which also have concentrations different from zero.*
5. The process of filling in the schedule table may be a long process, especially when there are many rows and/or many Blobs: during this process the software does not check the coherence of data entered, to avoid continuous interruptions for the user. A global check is performed only when the user gives a **Save** or **Calibrate** command: if some concentration is not coherent with the preceding schema, a message error is shown, giving the user the possibility to correct the data. When everything is right, the software accepts the schedule table, and redraws it using the following coloring model:
 - *Sample* Blob IDs (i.e., cells with zero concentrations), are shown with YELLOW background;
 - *Standard* Blob IDs (i.e., cells with non-zero concentrations), are shown with GREEN background.
 6. 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 the sample chromatograms and *fills the table with the calculated concentration values.*

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.

The various steps of the process are shown in Figure 5.2, Figure 5.3, Figure 5.4.

5.2.1 The Context Menu of the Schedule Table

When the number of the cells of the Schedule Table is large, the work of manually providing all values can be a long and tiring activity. In order to simplify this activity, especially when there are replications of the values, the user can use a special *context menu*, which allows some *copy and paste operations on cells and rows*. The context menu is activated by clicking with the right button of the mouse on a given cell; the menu shows the following items:

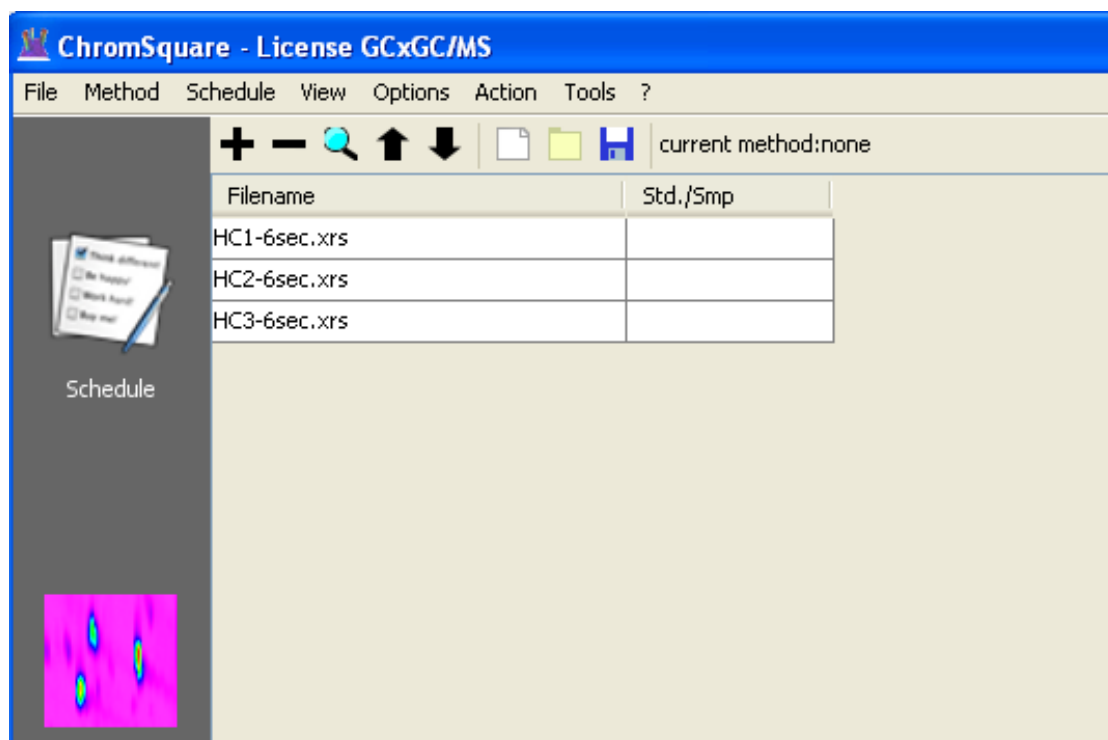


Figure 5.2: The Schedule Panel before loading a Method

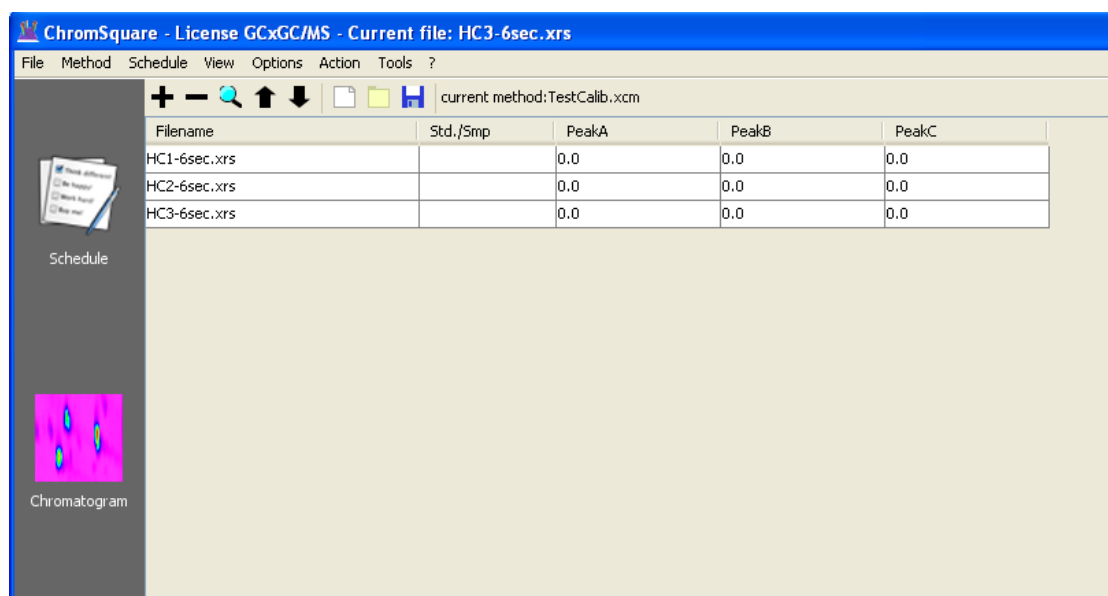


Figure 5.3: The Schedule Panel after loading a Method

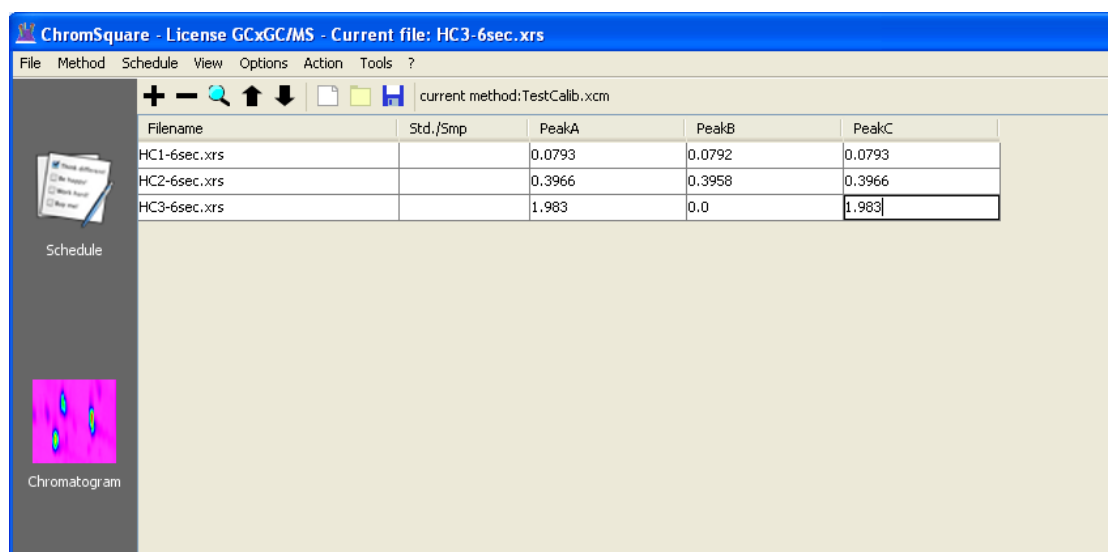


Figure 5.4: The Schedule Panel after the assignment of the Concentrations

- **Copy Cell:** the value of the currently selected cell is copied to the clipboard.
- **Paste Cell:** the value stored in the clipboard is copied to the currently selected cell.
- **Fill Row (Only null values):** the value stored in the clipboard is copied in the cells of the currently selected row which have values equals to zero.
- **Fill Row (All values):** the value stored in the clipboard is copied in all cells of the currently selected row.

The context menu is shown in Figure 5.5:

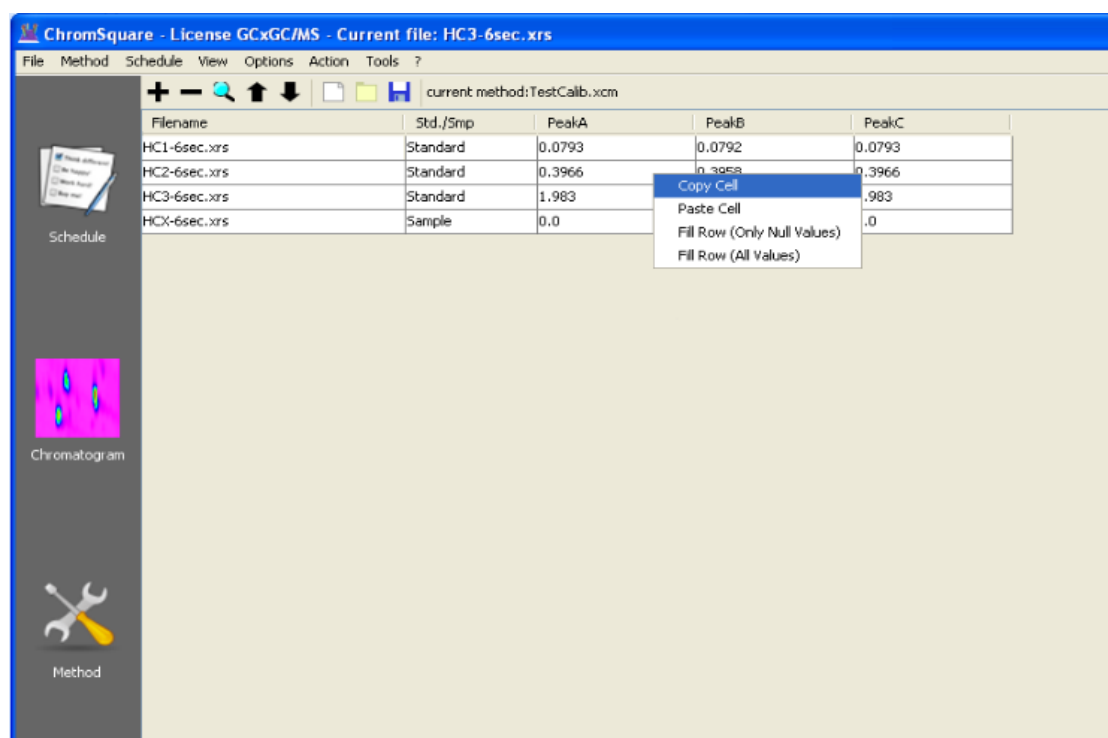


Figure 5.5: The context menu of the Schedule Table

Chapter 6

Method Panel

6.1 Panel contents

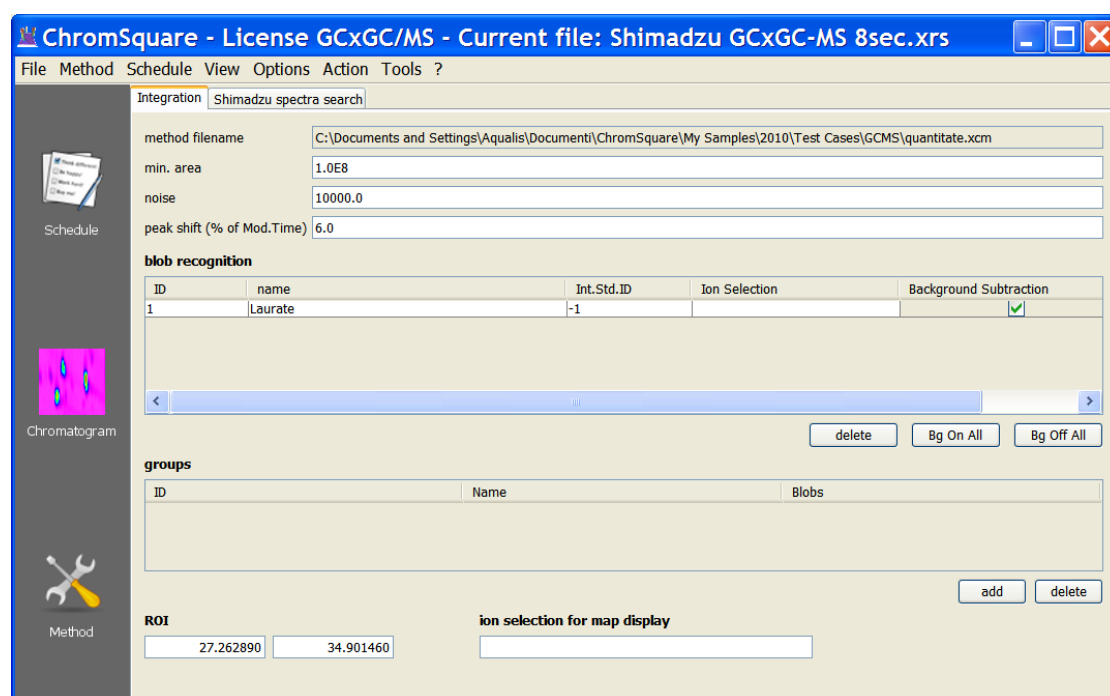


Figure 6.1: The Method Panel - Integration Page

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 one or more pages (*tabs*): the main page

Integration, which is always present, and optional pages depending on the purchased license (Shimadzu Spectra Search page and NIST Spectra Search page).

6.1.1 Integration page

The Integration page is shown in Figure 6.1.

Its components are described in the following paragraphs.

6.1.2 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**.

6.1.3 Method Parameters

This section contains three parameters; these 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.
- **Peak shift:** percent value per for the peak shift tolerance.

6.1.4 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.

The Table contains:

- a row for each Blob ID;
- the following five columns: **ID**, **name**, **Int.Std.ID**, **Ion Selection**, **Background Subtraction**.
 1. The first column contains the ID of the Blobs; normally, the ID is generated automatically.
 2. The second column contains the names of the Blobs; the names are normally assigned by the user in the Blob Definition procedure (see 7.2.4.2). 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.
 3. The third column 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.
 4. The fourth column is used to specify Ion Selection in the case of chromatograms generated by mass spectrometers; see Chapter 10.6 for details.
 5. The fifth column 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.

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 7.2.4.2).

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

6.1.5 Buttons for Blob ID Table management

Below the Blob ID Table there are 3 buttons, to be used for table editing:

- Button **delete**: 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 **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.

6.1.6 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.

6.1.7 Buttons 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 .

6.1.8 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 7.2.4.3).

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).

6.1.9 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 10.6).

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

6.2 Shimadzu Spectra Search page

The window for the **Shimadzu Spectra Search** is shown in the Figure 6.2:

In order to perform the Shimadzu spectra search, GCMSsolution, LCMSsolution or LabSolutions must be installed. Please read the technical note (Chapter 11.1) in order to correctly set up the Shimadzu software.

6.2.1 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.

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

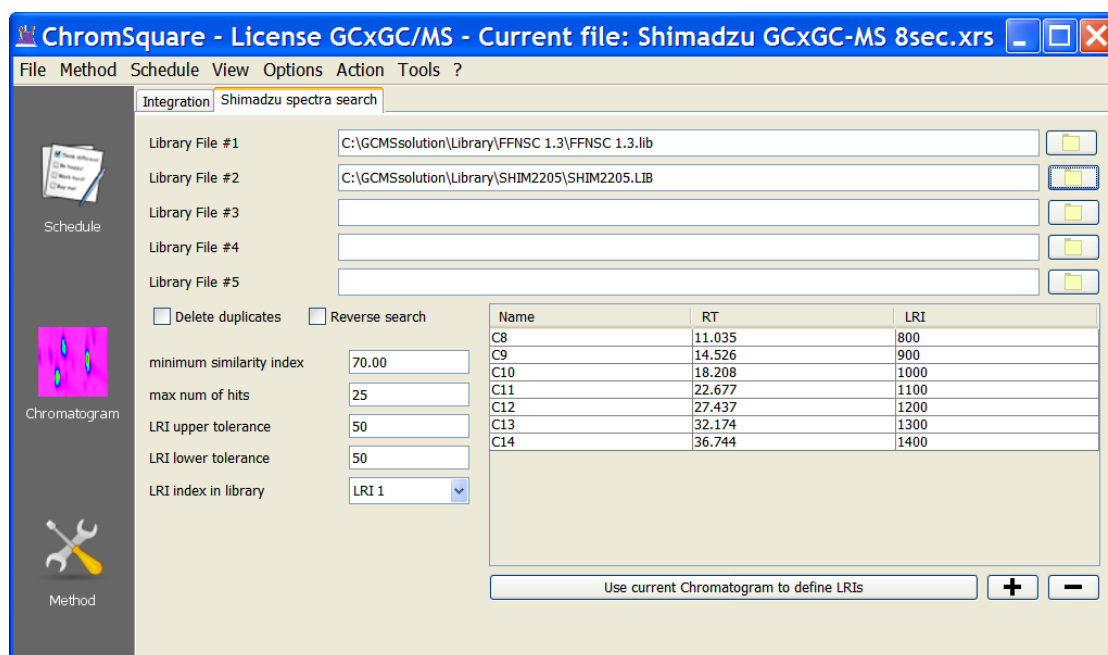


Figure 6.2: The Method Panel - Shimadzu Spectra Search Page

6.2.2 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.

6.2.3 Reverse search

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

6.2.4 LRI upper tolerance-lower tolerance

When using *Linear Retention Indexes* (LRI), the result set is filtered to the only hits that match the LRI within a *range* defines by these two numbers.

6.2.5 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.

6.2.6 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.

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. All the Blobs that are outside the RT interval between the first and the last entry of the table are assigned LRI equal to 0.

Chapter 7

Chromatogram Panel

7.1 Panel Contents

The Chromatogram Panel is made up of *two windows* horizontally aligned (that is, one above the other), and a *status bar* in the lower part of the panel.

- The *upper* window shows the Chromatogram as a colored Map, also called *Map Plot* or *Contour Map*; this window corresponds to the 2D representation of the chromatogram; it is described in Section 7.2.
- The *lower* window contains various *sub-windows* or *pages* that can be selected using the upper *tabs*:
 - *Graphical Parameters* page (see Section 7.3); this page contains:
 - * the *Color Map Panel*;
 - * the *Navigation Panel*.
 - *Chromatogram* page (see Section 7.4).
 - *Spectrum* page (see Section 7.5).
 - *Data Analysis* page (see Section 7.6).
 - *Grouping* page (see Section 7.7).
- The *status bar* (see Section 7.8) displays information about the current position of the mouse cursor inside the upper Map Plot.

The upper and the lower windows are separated by an *horizontal floating bar*, that is a bar that can be dragged up and down with the mouse, allowing the resize of the two windows according to current needs of the user.

7.2 The Map Plot Window

7.2.1 Features

The upper Map Plot window 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 than 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) 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*;

7.2.2 Selection

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

The user can then select through the mouse a smaller a smaller region of the Contour Map in a way that it will be represented in the whole Map Plot window: 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 7.2.3.

The selection of 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 7.2.4 for a full description of this menu.

The Figure 7.1 shows a Map Plot selected region and the Plot Context Menu.

7.2.3 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 Panel*) of the lower panel (see 7.3.2 for a full description).

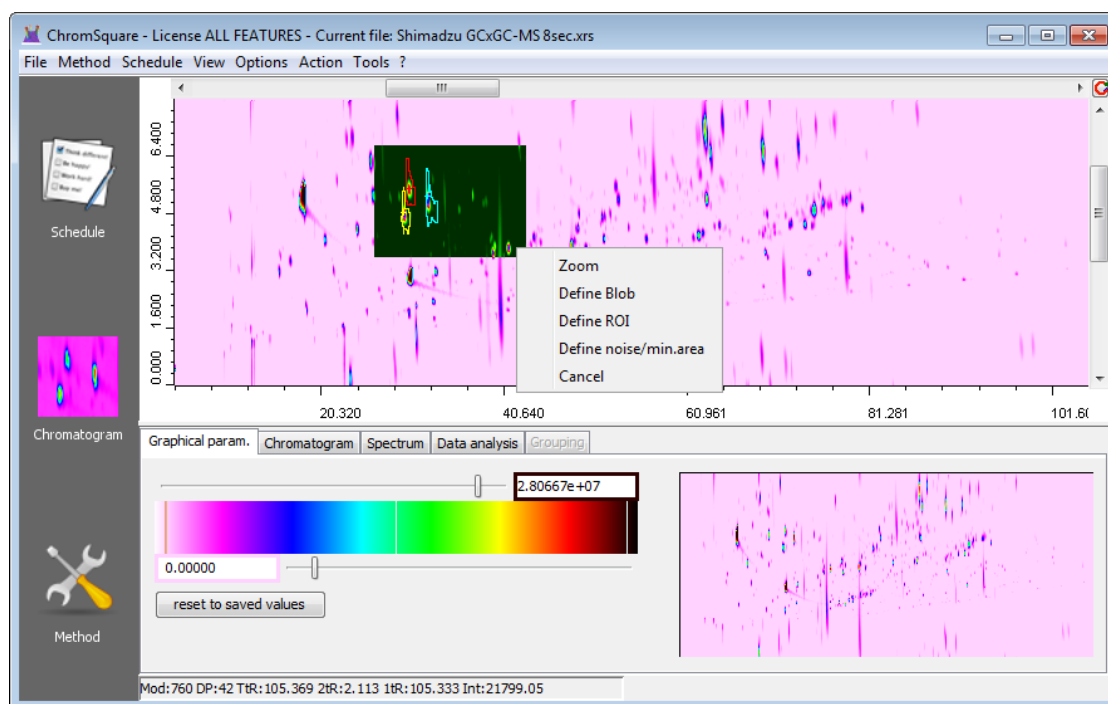


Figure 7.1: Map Plot - Context Menu

The Figure 7.2 shows the Map Plot after Zoom In operation, and the representation of the zoomed region in the Zoom Mapping facility. This is the same setting of 7.1 after that the first menu item (Zoom) has been selected.

7.2.3.1 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.

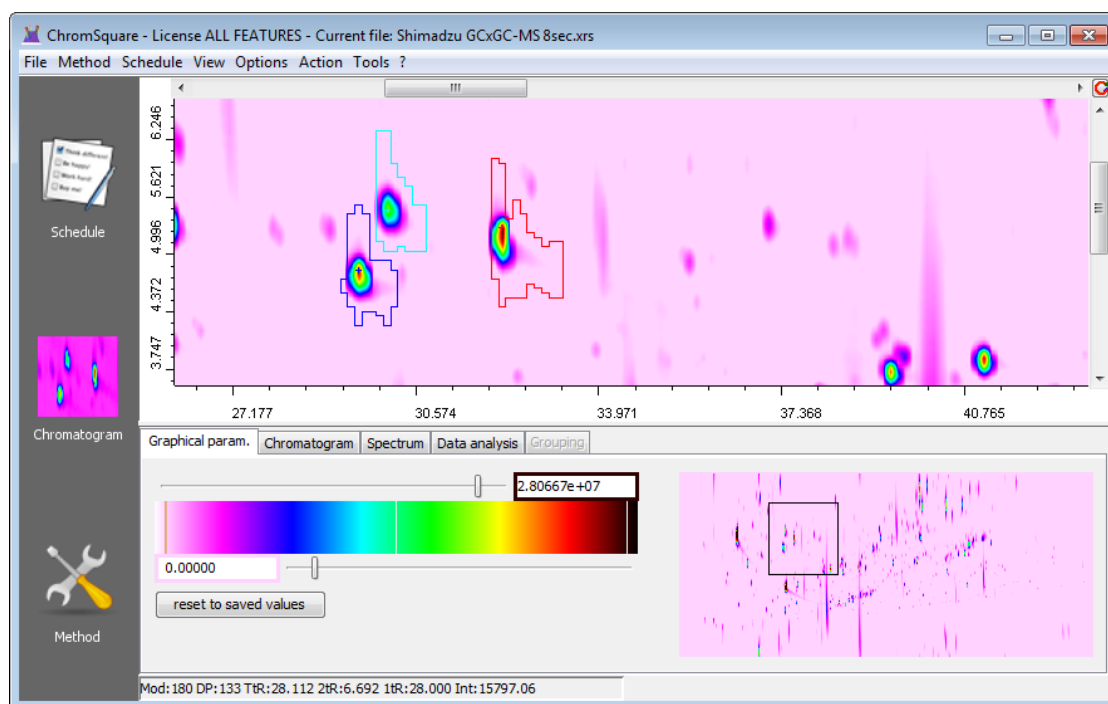


Figure 7.2: Map Plot - Zoomed Region

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

7.2.3.2 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.

7.2.3.3 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.

7.2.4 Map Plot Context Menu

The context menu is displayed after that a rectangular region of the Map Plot has been selected. This rectangular region represents the *domain* to which the operation of the selected menu item will apply.

The context menu (shown in Figure 7.1) contains the menu items described in the following Sections.

7.2.4.1 Zoom

A *Zoom In* operation will be performed on the selected region. See 7.2.3.1 for a full description of Zoom In operation.

7.2.4.2 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 7.2.2.
- 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** to confirm the assignment, or **Cancel** to delete the current assignment.

The window is shown in Figure 7.3.

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 10.3 for details). A rectangular region becomes in this way a particular case of a polygonal one.

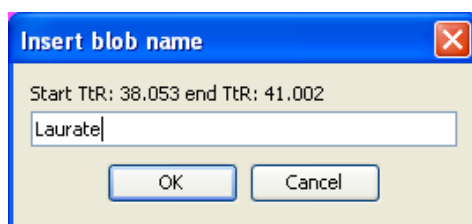


Figure 7.3: Insert Blob Name window

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 Panel¹.

Figure 7.4 shows the Method Panel after that the new Blob ID has been defined.

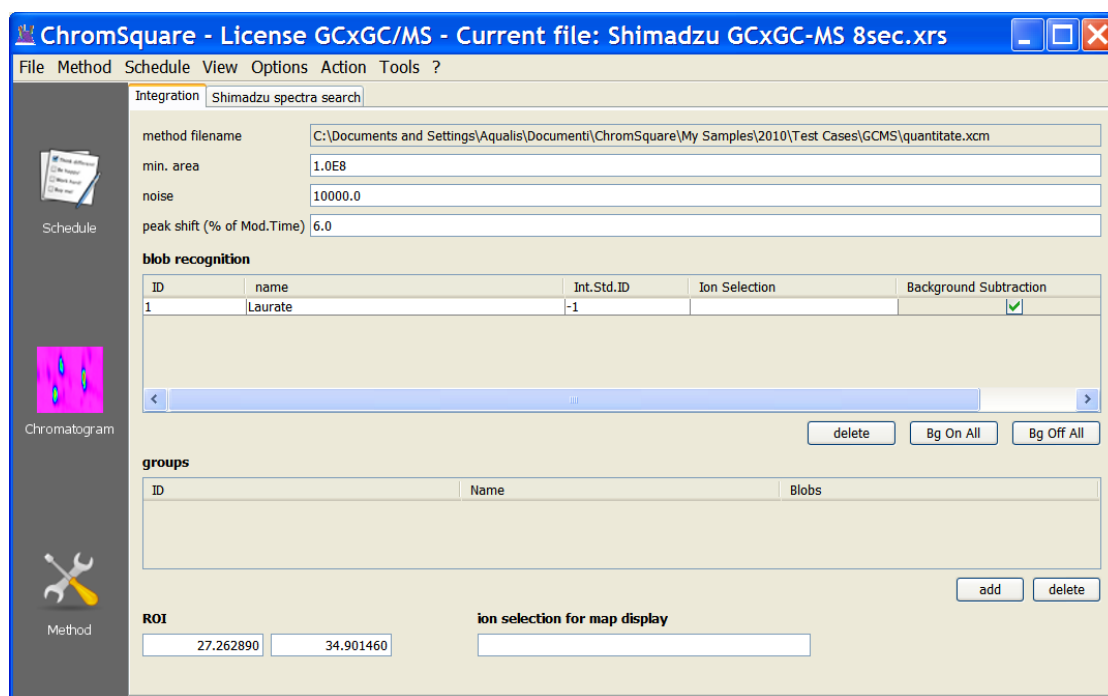


Figure 7.4: The Method Panel with the new Blob ID

¹The 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

7.2.4.3 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.

7.2.4.4 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 6.1.3).

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.

7.2.4.5 Cancel

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

²The 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.

7.3 The Graphical Parameters Page

The Graphical Parameters page is divided into two panels:

- the left panel is used for defining the Color Mapping;
- the right panel is used for representing (and changing) the zoomed region with respect to the overall chromatogram; it is called the Navigator Panel.

7.3.1 The Color Mapping Panel

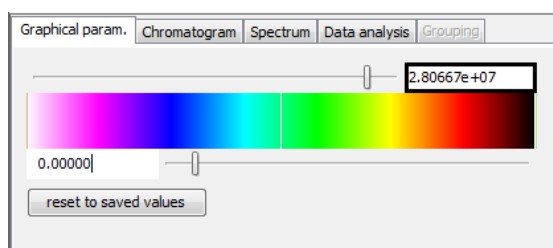


Figure 7.5: Graphical Parameters Page

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 7.2.

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 7.5. 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 7.3.1.2;
- 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 7.3.1.2;
- a *Reset* button; this allow to restore the previously saved values of all scales.

7.3.1.1 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).

7.3.1.2 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*.

7.3.1.3 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.

7.3.2 The Navigator Panel

The *Navigator Panel* 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 Plot window (even if with less details and different size). When the zoom is activated, the Navigator Panel window shows the chromatogram with a *rectangle* inside it, representing the *zoom area* which is displayed enlarged in the main Map Plot window.

A sample is shown in Figure 7.6.

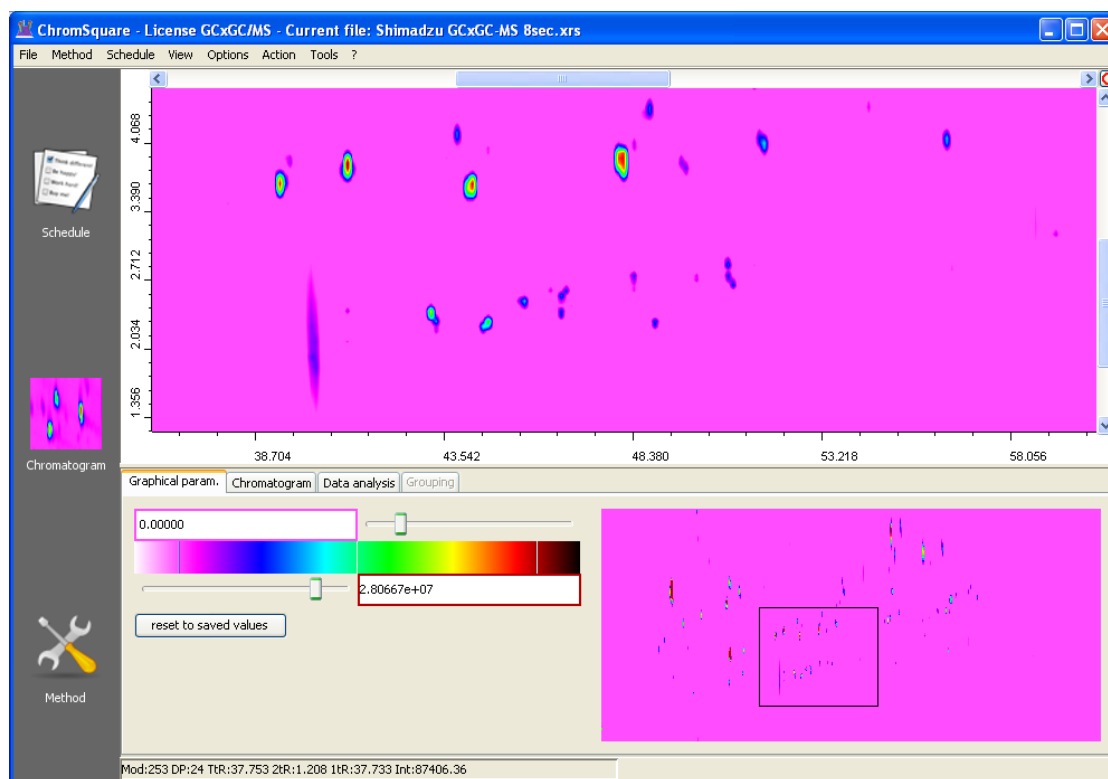


Figure 7.6: Navigator Panel with Zoomed Area

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 Panel;
- Operating on the *scroll bars* of the main Map Plot.

7.3.2.1 Moving the zoom area with the Navigator Panel

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

- by *clicking on the center* of the new position in the Navigator Panel; 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.

7.3.2.2 Moving the zoom area with the Map Plot 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 Plot.

The sliders show the current *position* and *sizes* of the zoom area with respect to the overall Map Plot view. The fraction of the sizes of the slider with respect to the corresponding sizes of the Map Plot 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 Panel 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`).

7.4 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 Plot.

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

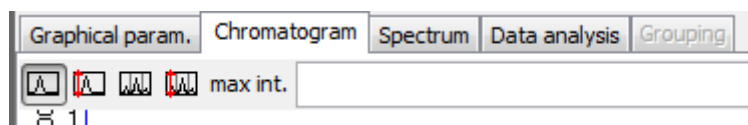


Figure 7.7: The Toolbar of the Chromatogram page

7.4.1 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 Plot), on a time domain that is a *fraction* of the *modulation time*, corresponding to the vertical zoom interval in the 2D Map Plot

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.**, either directly writing a new value in the text field, or using the two small arrows to quickly change it.

See Figure 7.8.

7.4.2 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

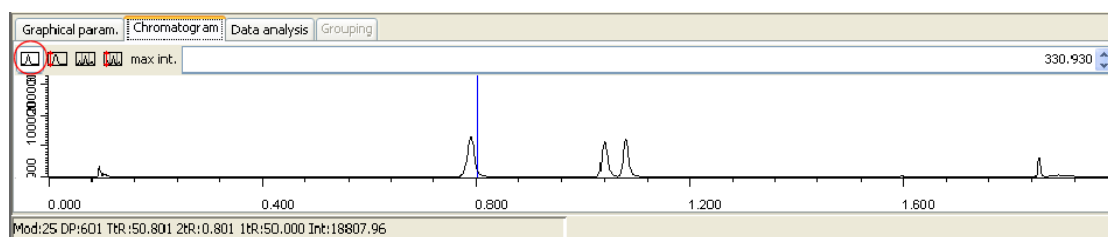


Figure 7.8: Single modulation in zoom interval

position in the 2D Map Plot), on a time domain corresponding to the *whole modulation time*.

More precisely:

- Abscissa axis is *retention time*, ranging from 0 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 7.4.1.

See Figure 7.9.

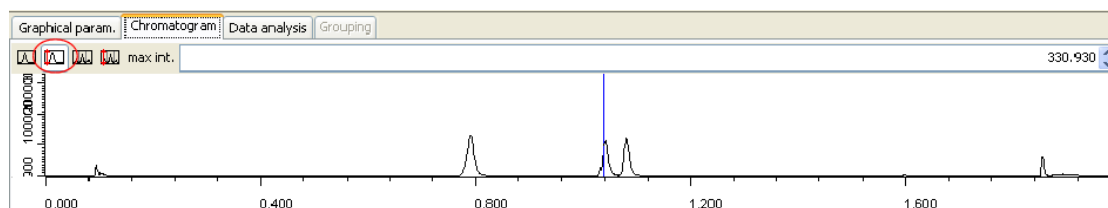


Figure 7.9: Single modulation in whole modulation interval

7.4.3 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 Plot.

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 7.4.1.

See Figure 7.10.

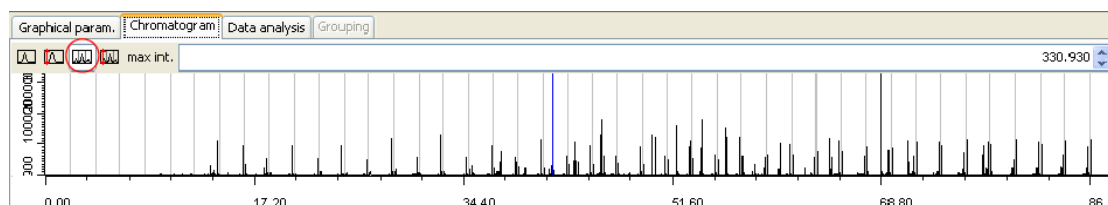


Figure 7.10: Multiple modulation in zoom interval

7.4.4 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 7.11.

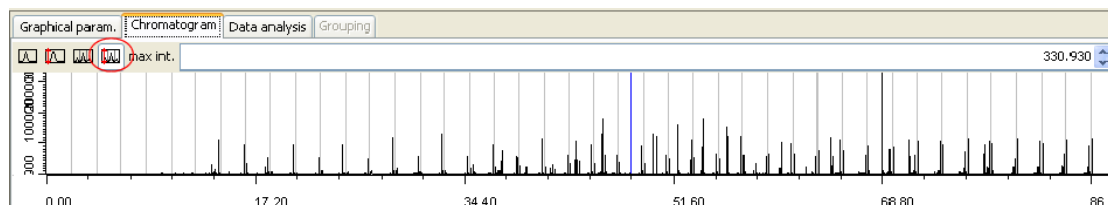


Figure 7.11: Multiple modulation in whole modulation interval

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 7.4.1.

7.5 The Spectrum page

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

Mass Spectra (when available) can be viewed by selecting the page **Spectrum** in the lower panel. As the user moves the mouse in the upper window, the spectrum quickly changes in the lower window, following the various modulations (like in the case of Chromatogram page, see 7.4).

A sample of Spectrum page is shown in Figure 7.12.

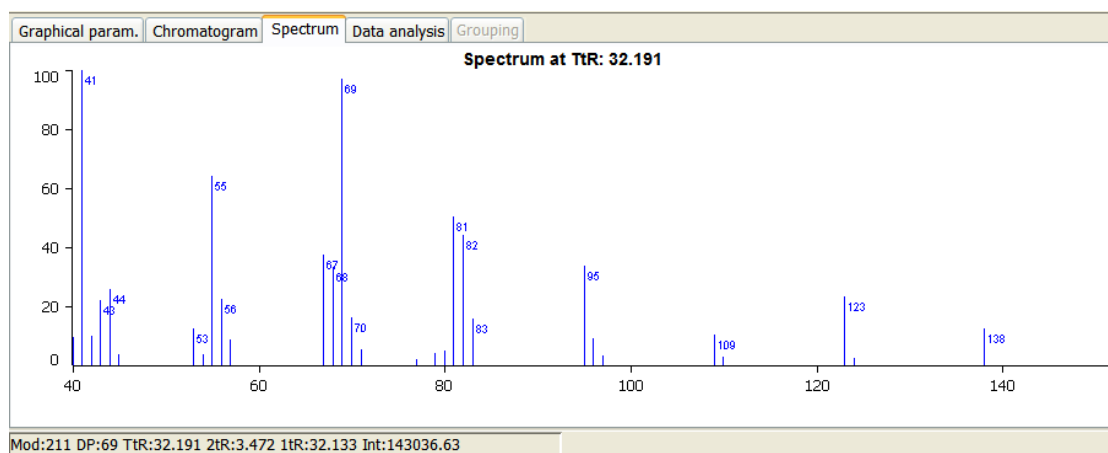


Figure 7.12: The Spectrum page

7.6 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 7.13. Since the table is rather large, the Figures 7.14 and 7.15 report an enlarged view for a better readability.

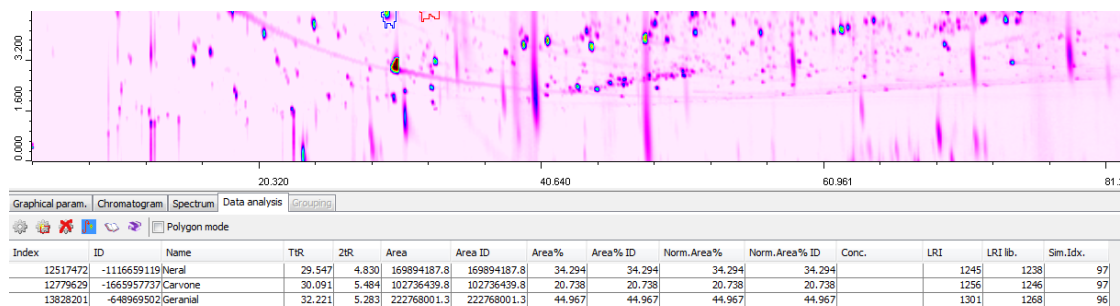


Figure 7.13: Data Analysis page - Overall view

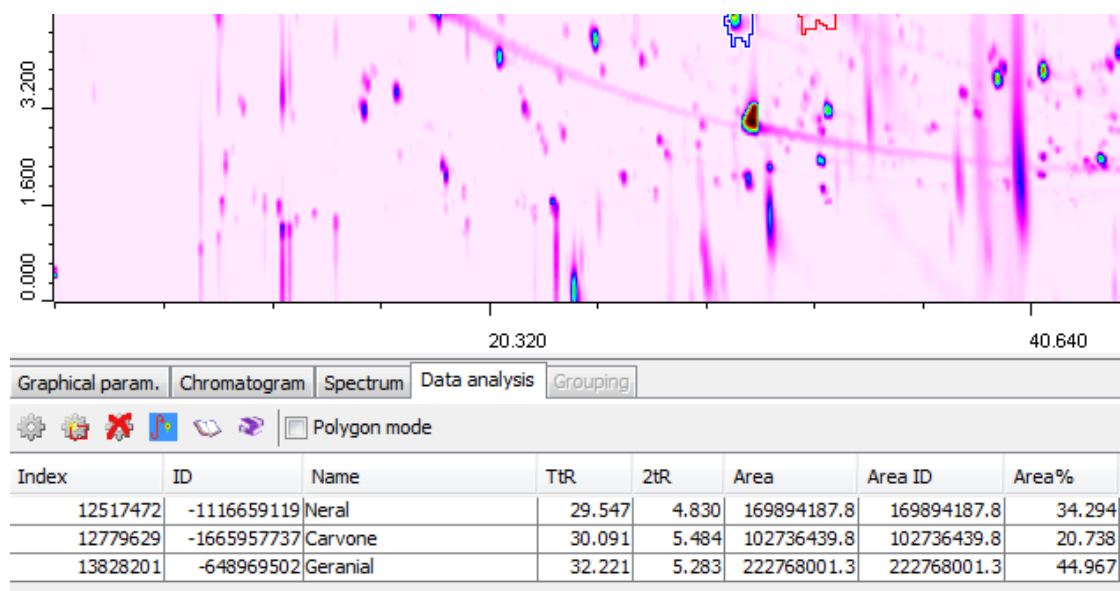


Figure 7.14: Data Analysis page - Left view

7.6.1 The Data Analysis table

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

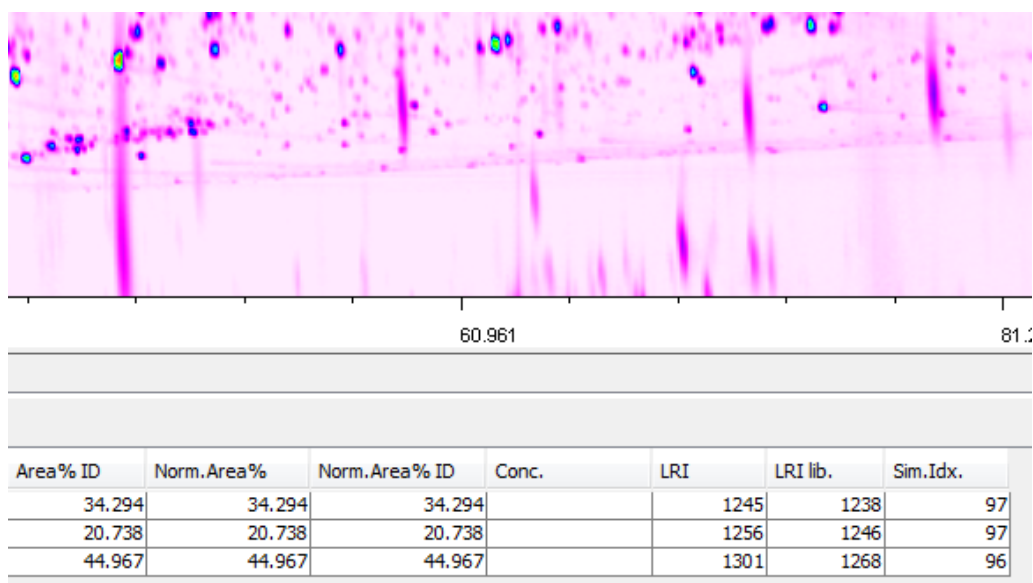


Figure 7.15: Data Analysis page - Right view

The different columns have the following meaning³:

1. **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.
2. **ID.** The Blob ID, see 3.1.6.
3. **Name.** Name of the Blob.
4. **TtR.** Total retention time of the Blob.
5. **2tR.** Retention time in 2nd dimension of the Blob.
6. **Area.** Area of the “individual” Blob.
7. **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.
8. **Area %.** Area percent of the individual Blob, calculated with respect to the sum of the areas of all Blobs.

³According 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.

9. **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.
10. **Normalized Area %.** Area percent of the individual Blob, normalized with respect to the sum of the areas percent of all *identified* Blobs.
11. **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.
12. **Concentration.** This column will be filled after quantitation.
13. **LRI.**
14. **LRI lib.**
15. **Sim. Index.**

7.6.1.1 A Grouping sample

The Figure 7.16 shows an example of 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 than 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.

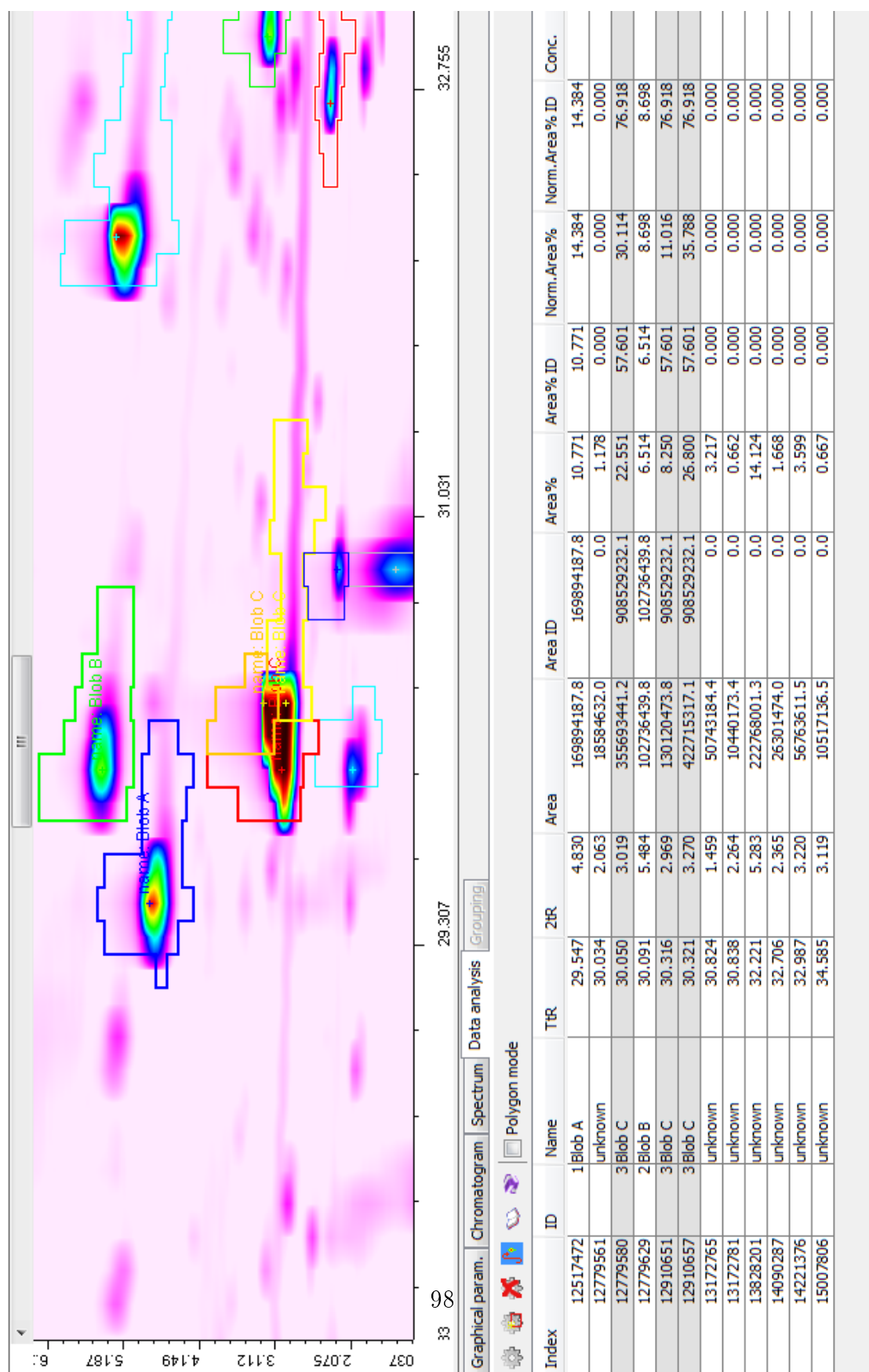


Figure 7.16: Data Analysis page - Example showing ID Grouping case

7.6.2 The Data Analysis Toolbar

The Toolbar is shown in Figure 7.17.

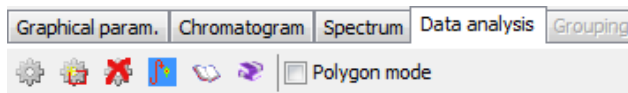


Figure 7.17: Data Analysis page - The Data Analysis Toolbar

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 4.6.13.
5. (*book*) **Search selected Blob** in spectra library. It corresponds to the menu Action command **Search selected Blob** of Section 4.6.11.
6. (*book collection*) **Search all Blobs** in spectra library. It corresponds to the menu Action command **Search all Blobs** of Section 4.6.12.
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 7.2.3 and 7.2.4.2); if checked, the Polygon mode is active (see Section 10.3)

7.6.3 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 Plot with *enhanced boundaries*, like in Figure 7.18.

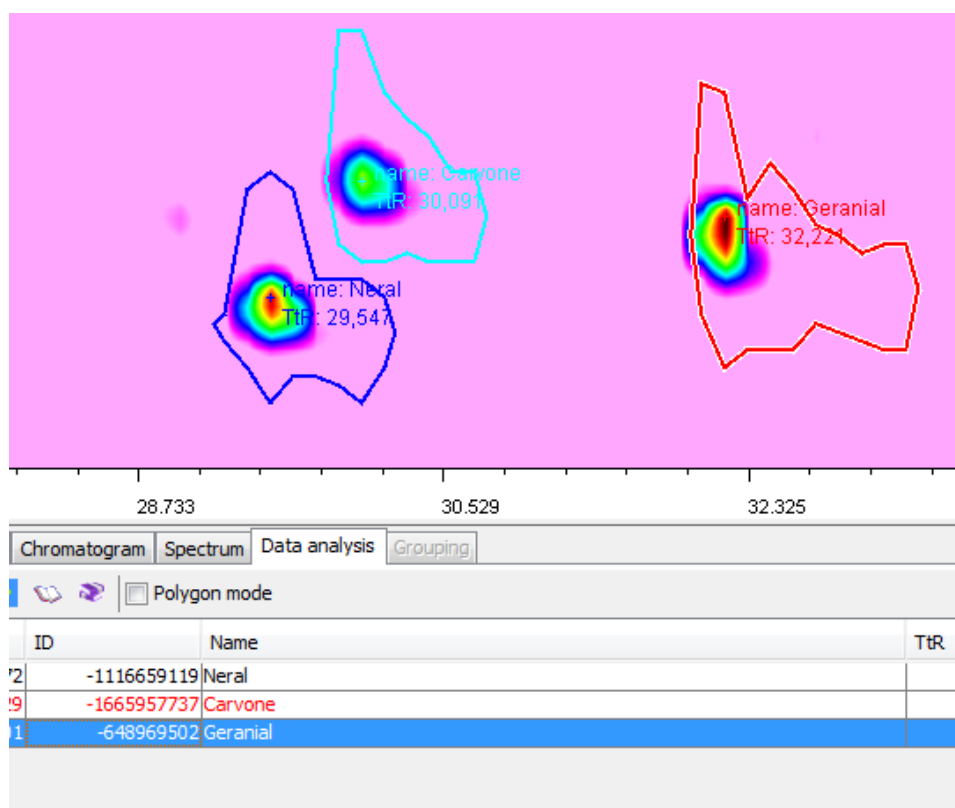


Figure 7.18: Enhanced representation of the selected Blob

The context menu is represented in Figure 7.19. The various items are described in the following sections.

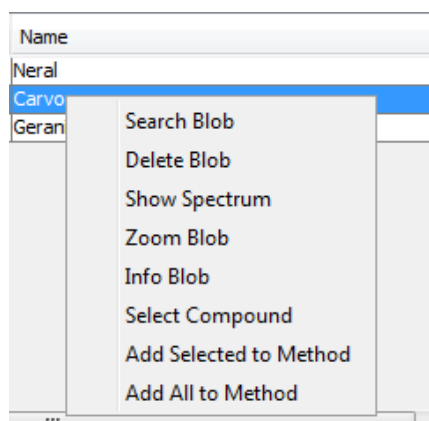


Figure 7.19: Data Analysis page - The Data Analysis Context Menu

7.6.3.1 Search Blob

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

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

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

7.6.3.2 Delete Blob

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

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

7.6.3.3 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, which displays the Mass Spectrum of the currently selected Blob. A sample is given in Figure 7.20.

The window contains two Spectrum Plots:

1. The upper plot displays the mass spectrum at the *top point* of the Blob;

2. the lower plot displays the *average spectrum*, computed as the average of all spectra corresponding to the chromatographic points of the Blob;

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

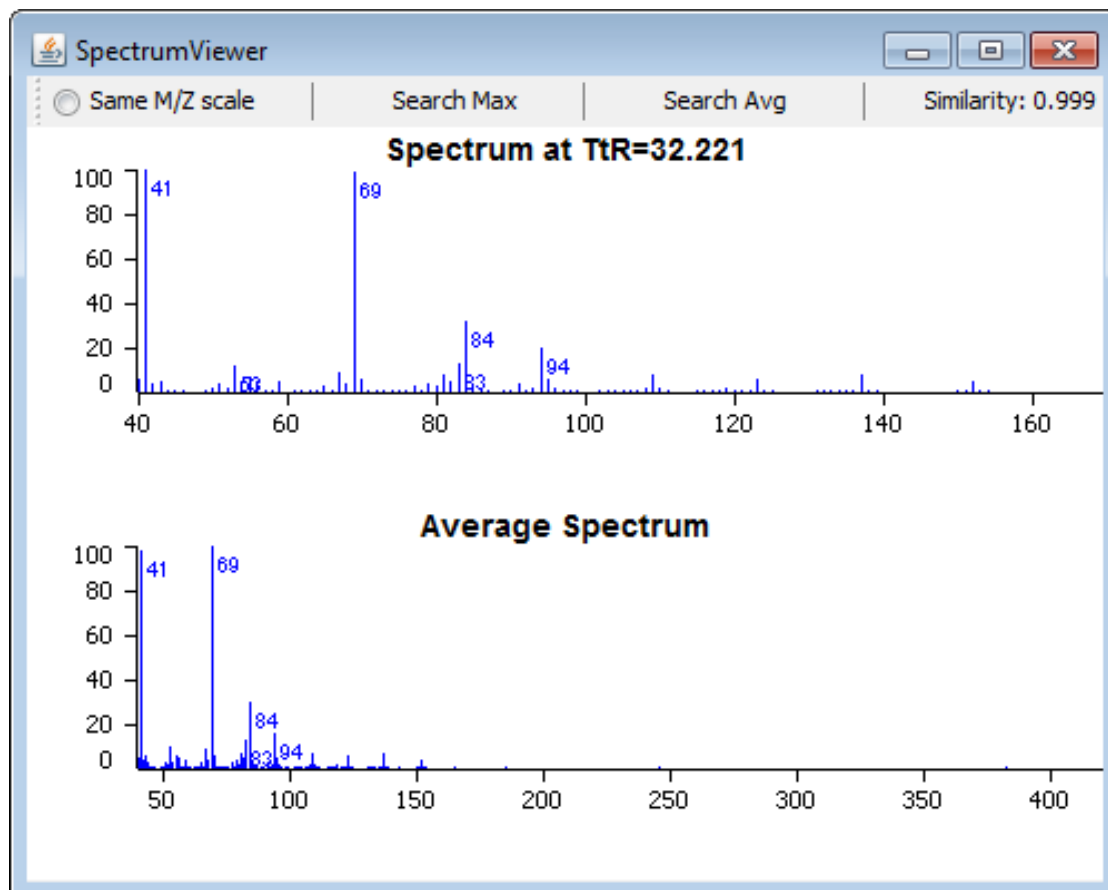


Figure 7.20: Show Spectrum window

7.6.3.4 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 7.21.

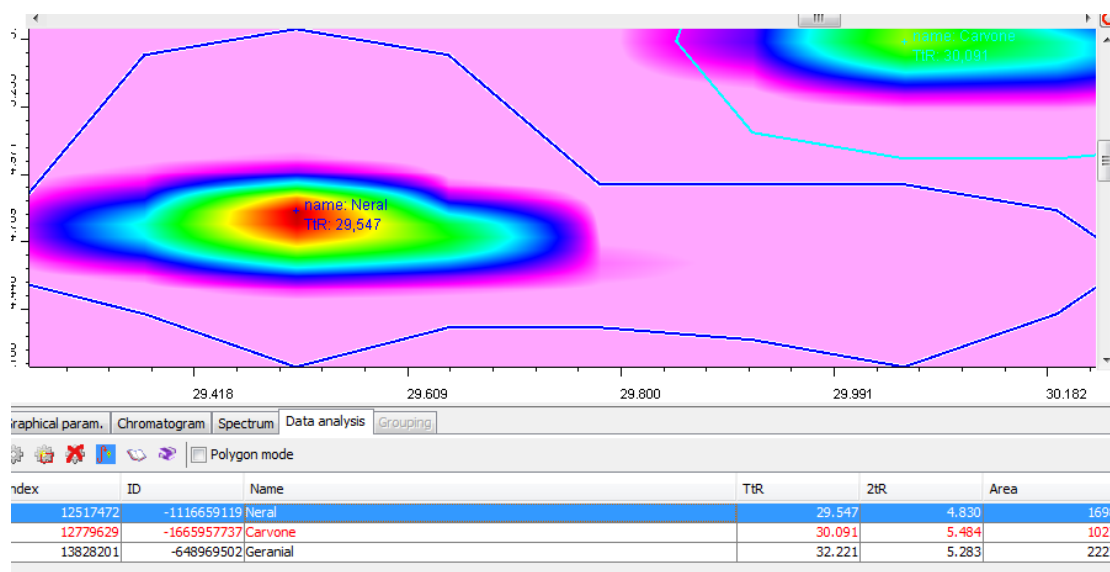


Figure 7.21: A full zoomed Blob

7.6.3.5 Info Blob

The command displays a window which reports a description of the Blob, including a list of all peaks corresponding to the various modulations, with a very detailed description for each of them.

A sample is shown in Figure 7.22.

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

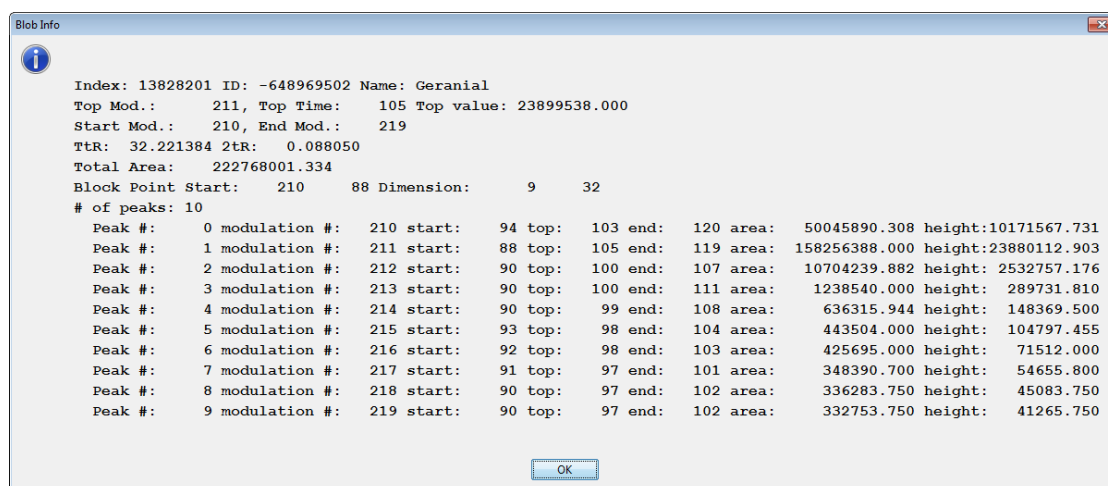


Figure 7.22: The window displaying Blob Info

7.6.3.6 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 7.6.3.1 or the main menu command **Search All Blobs** of Section 4.6.12).

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 7.23.

7.6.3.7 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).

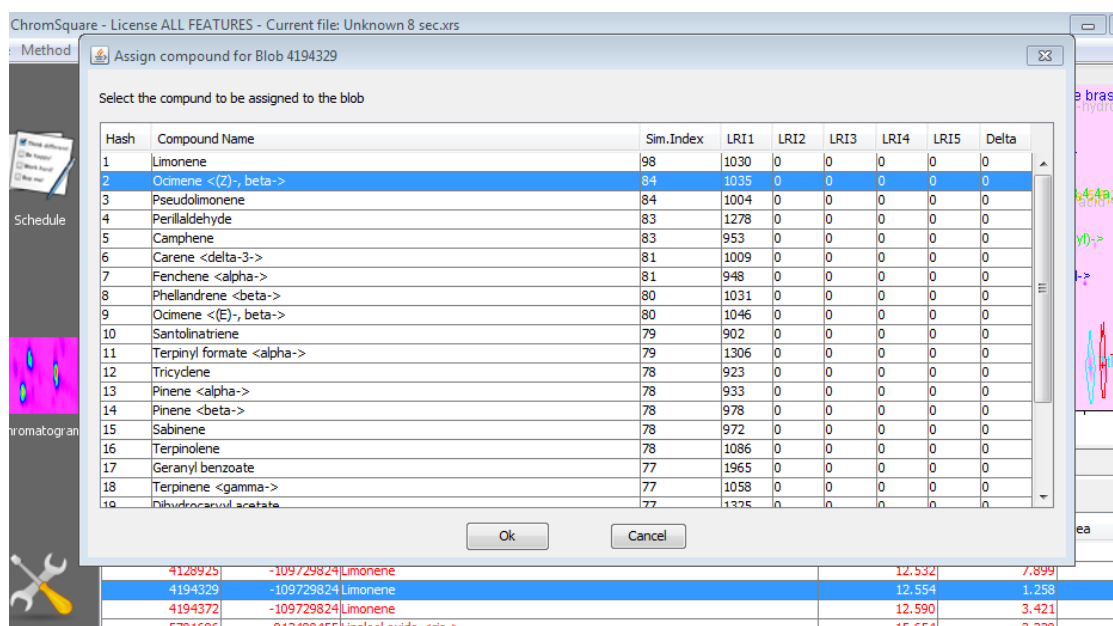


Figure 7.23: The Assign Compound dialog box

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 7.6.1).
- 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*.

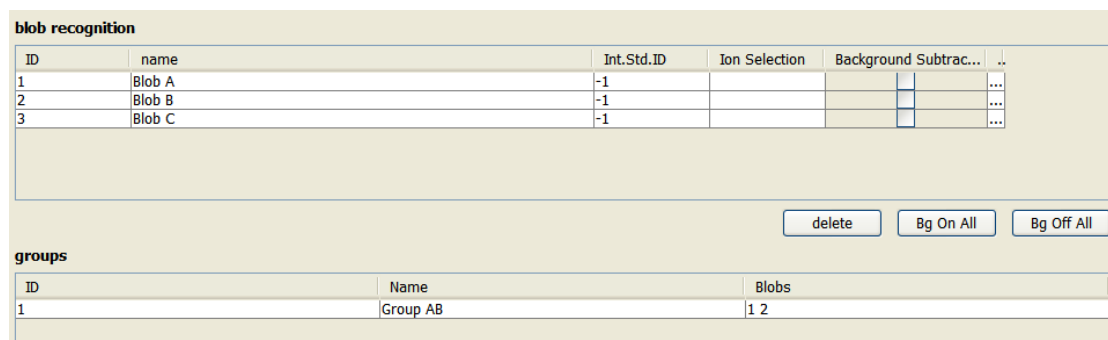
7.6.3.8 Add All to Method

The command automatically performs the procedure of adding a Blob to the Method (described in the previous Section 7.6.3.7) 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.

7.7 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.



The screenshot shows two panels. The top panel, titled 'blob recognition', contains a table with columns: ID, name, Int.Std.ID, Ion Selection, Background Subtrac..., and a dropdown menu. It lists three blobs: Blob A, Blob B, and Blob C, each with an Int.Std.ID of -1. Below the table are three buttons: 'delete', 'Bg On All', and 'Bg Off All'. The bottom panel, titled 'groups', contains a table with columns: ID, Name, and Blobs. It lists one group: Group AB, with Blobs 1 and 2.

ID	name	Int.Std.ID	Ion Selection	Background Subtrac...	..
1	Blob A	-1			...
2	Blob B	-1			...
3	Blob C	-1			...

ID	Name	Blobs
1	Group AB	1 2

Figure 7.24: Method Panel showing one Group

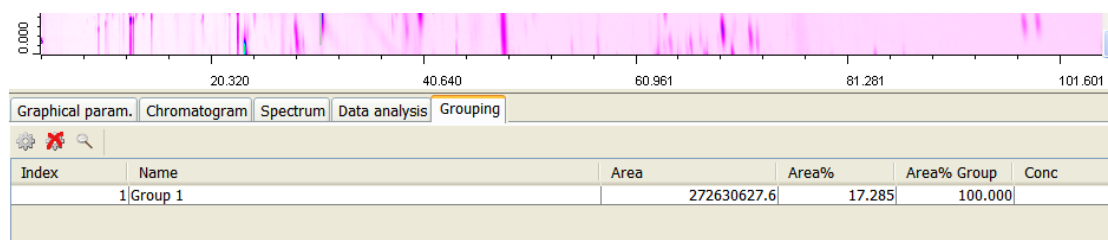


Figure 7.25: Grouping page - The results

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 7.24 shows a Method Panel with the definition of a Group. The example shown refers the same case described in Section 7.6.1.1: a Group, named “Group AB”, has been defined as the aggregation of Blob A and Blob B.

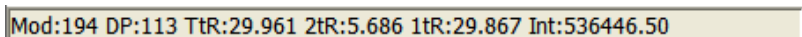
The Figure 7.25 shows the results of the integration in the Grouping page. Comparing the results with those shown in Figure 7.13, it can be seen that the Area of the Group AB is equal to the sum of Areas of Blob A and Blob B. The same for Area %.

7.8 The Status Bar

The Status Bar (see Figure 7.8) shows the current position of the mouse pointer inside the Map Plot window. 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: $TtR = 1tR + 2tR/60$



Mod:194 DP:113 TtR:29.961 2tR:5.686 1tR:29.867 Int:536446.50

Figure 7.26: Chromatogram Panel - The Status Bar

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 4.6.13.

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 6.
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.
 - **Peak shift:** value per cent for the *peak shift* tolerance.

In the case of a new Method, the three fields are initially filled with the value -1.0, which means that a default value is assumed for each parameter.

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

method filename	C:\ChromSquare\samples\Collaudo\Test 3.2.xcm
min. area	1.0e8
noise	10000.0
peak shift (% of Mod.Time)	6.0

Figure 8.1: The Method 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 7.6.2)

Figure 8.2 shows a samples of whole chromatogram integration.

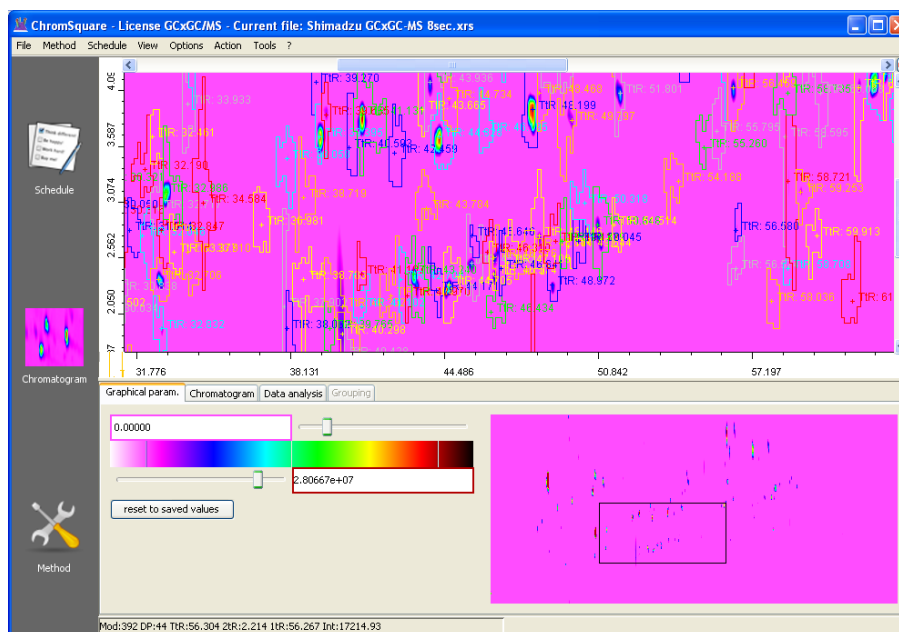


Figure 8.2: Qualitative Analysis: Whole Chromatogram integrated

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 6.1.8). To define a ROI, use the Map Plot Context Menu (see Section 7.2.4).
- 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 7.6.2)

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 7.2.3);
 - 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 7.3.1.

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 7.6.
- View the *linear chromatogram profile* in correspondence of the single Blobs, as described in Section 7.4.

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.

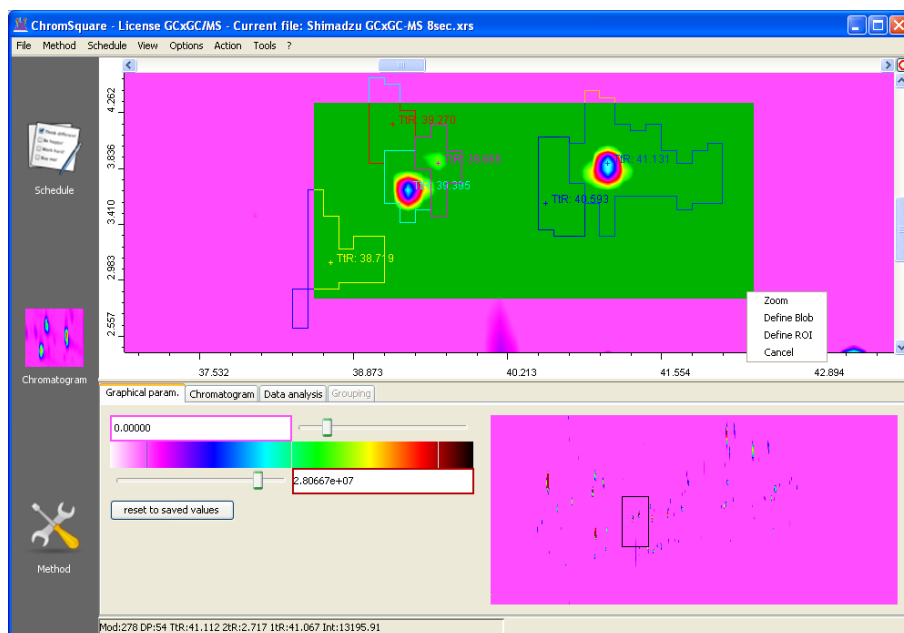


Figure 8.3: Qualitative Analysis: Define ROI



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 7.2.2 and 7.2.4.
- Enter the Blob name in the dialog box that will be displayed soon after, as described in Section 7.2.4.2.

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 10.3.

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 GCsolution(using Shimadzu Search engine).
- LCxLC version for Shimadzu LCMSsolution and LCsolution(using Shimadzu Search engine).
- LCxLC version for Shimadzu LCsolution(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 7.6.3.1);
- or the command **Search Selected Blob** (Section 4.6.11) of the **Action** main menu.

To search *all* Blobs:

- give the command **Search All Blobs** (Section 4.6.12) 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 7.6.3.6.

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 7.6.3.8.

Chapter 9

Quantitative Data Analysis

In this chapter it will be presented a summary of the operations of a routine *Quantitative Analysis*. The summary will be illustrated with frequent *screen shots*, which drive the user along the various steps of the procedure.

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 errors¹.

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.

In the directory shown in Figure 9.1 the files have been renamed according to the aforementioned rules.

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.

See Figure 9.2.

9.1.1.3 Add files to the Schedule

Use the **Plus Button** (that 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.

¹The 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.

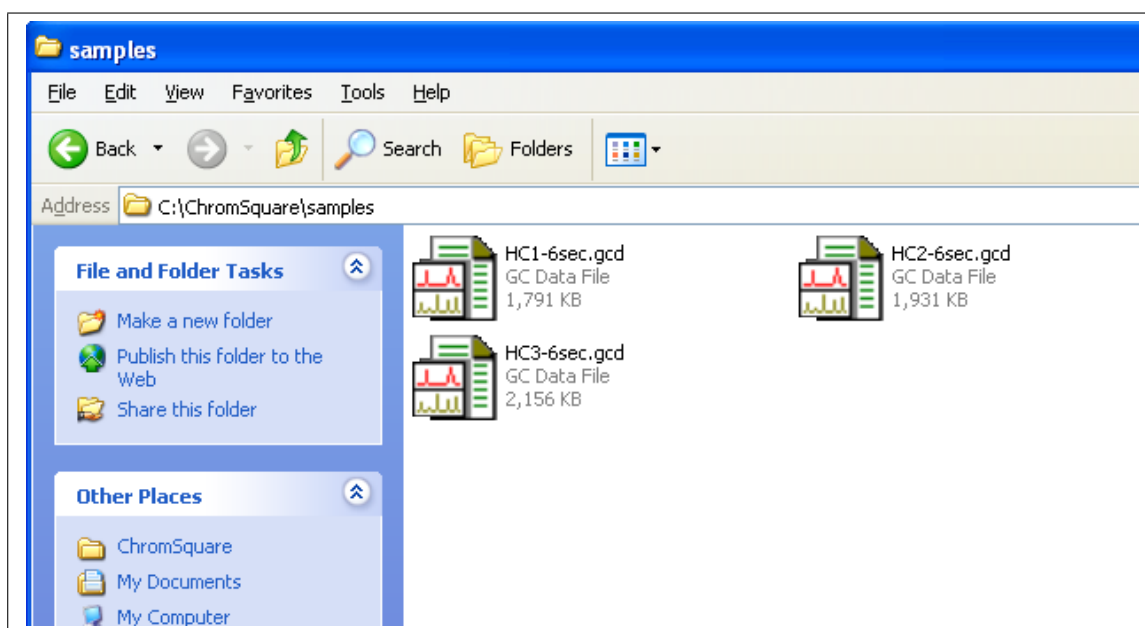


Figure 9.1: A list of files properly ordered for quantitative analysis

The operation is shown in Figure 9.3.

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.

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).

The Schedule Menu is shown in Figure 9.4.

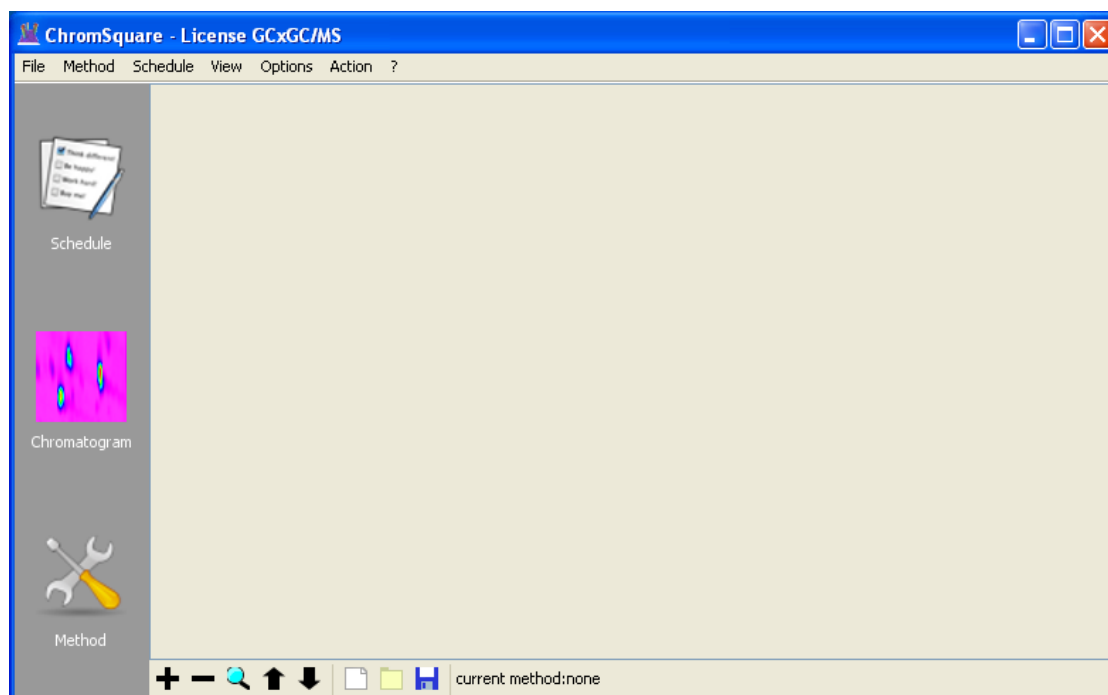


Figure 9.2: Initial Window for Quantitative Analysis

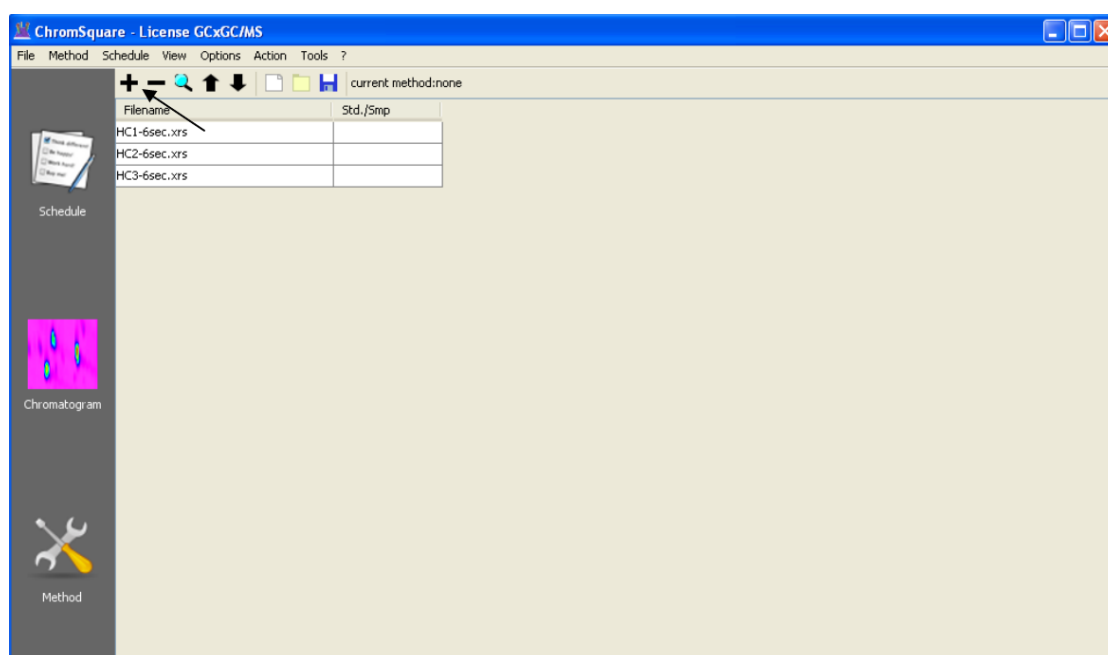


Figure 9.3: Schedule Panel with some Chromatograms added

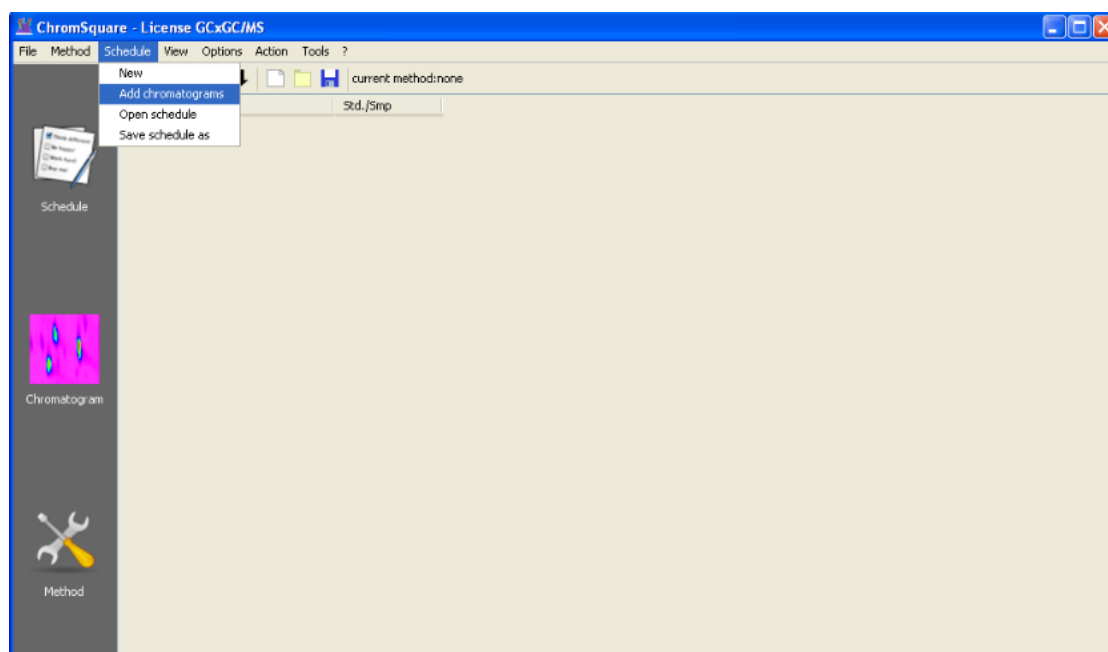


Figure 9.4: The Schedule Menu with “Add chromatograms” command selected

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 through the menu command **Method|New Method** (see Section 4.2.1).

Figure 9.5 shows the switch to Method Panel icon; Figure 9.6 shows the Open Method menu command.

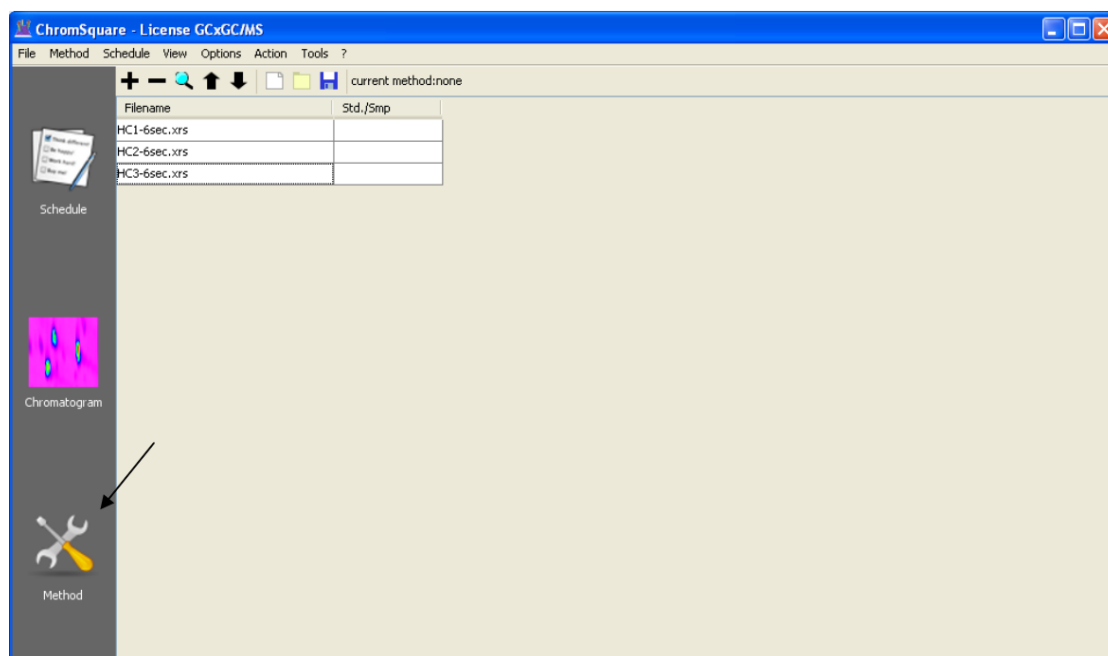


Figure 9.5: Switch to the Method Panel

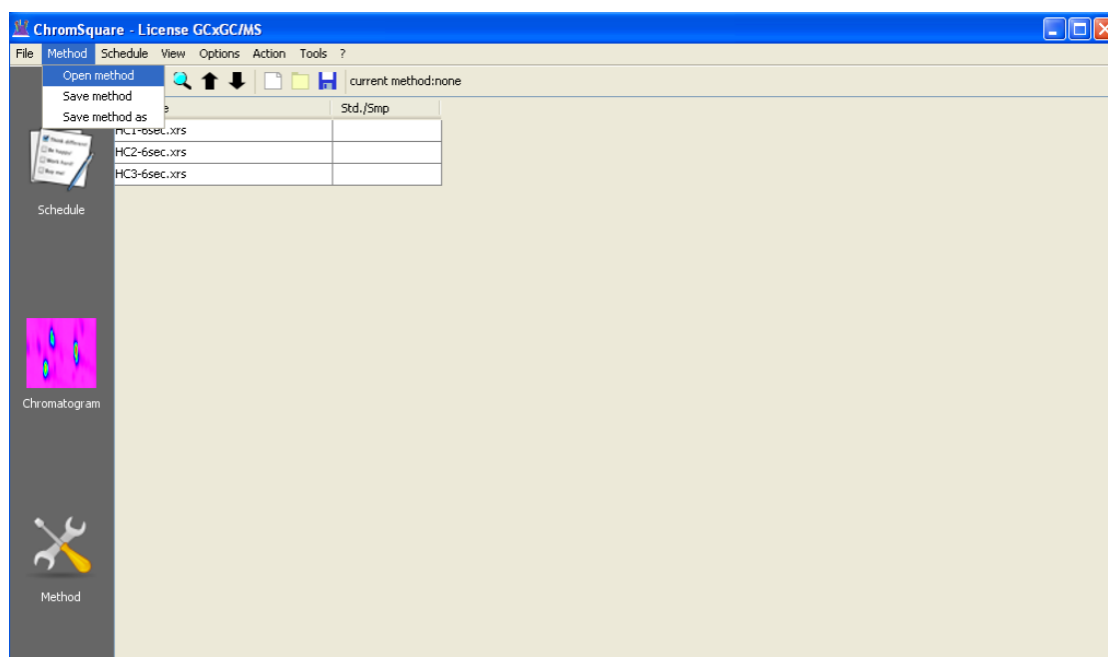


Figure 9.6: The Method Menu with “Open Method” command selected

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.

The Method Panel with an empty Method is shown in Figure 9.7.

9.1.2.2 Define Method Parameters

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

The Method Panel with these fields filled is shown in Figure 9.8. In this case, the numbers 100000, 10000, 6 have been used for the three parameters.

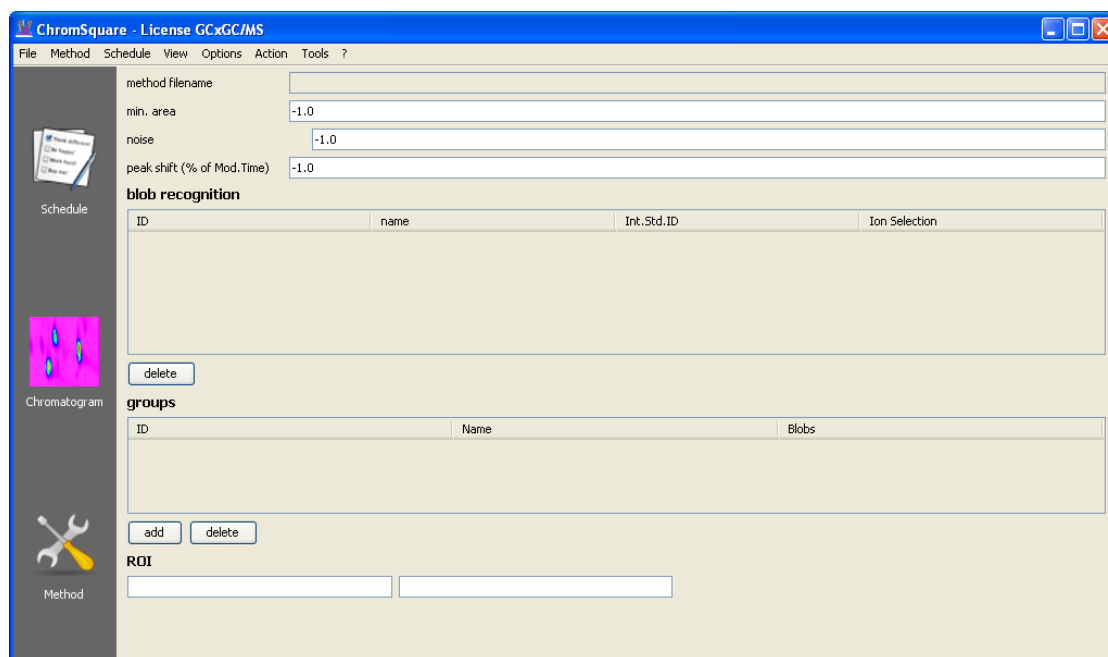


Figure 9.7: The Method Panel showing a new empty Method

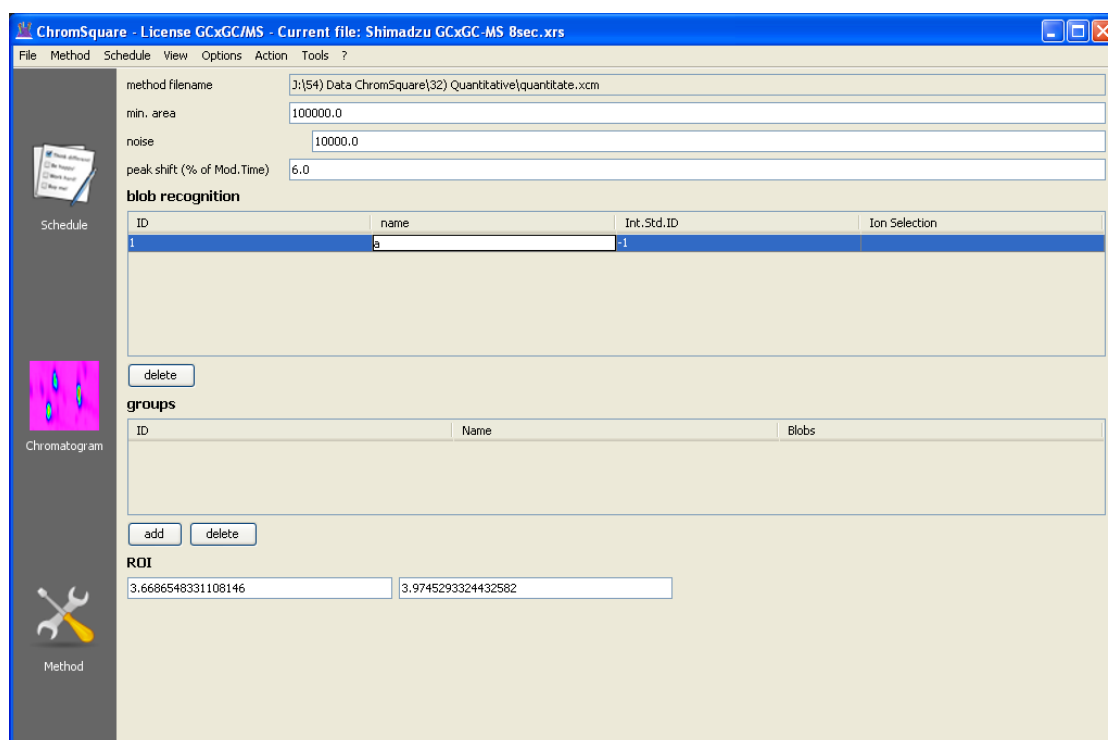


Figure 9.8: The Method Panel with some parameters entered

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.

The icon is pointed out in Figure 9.9.

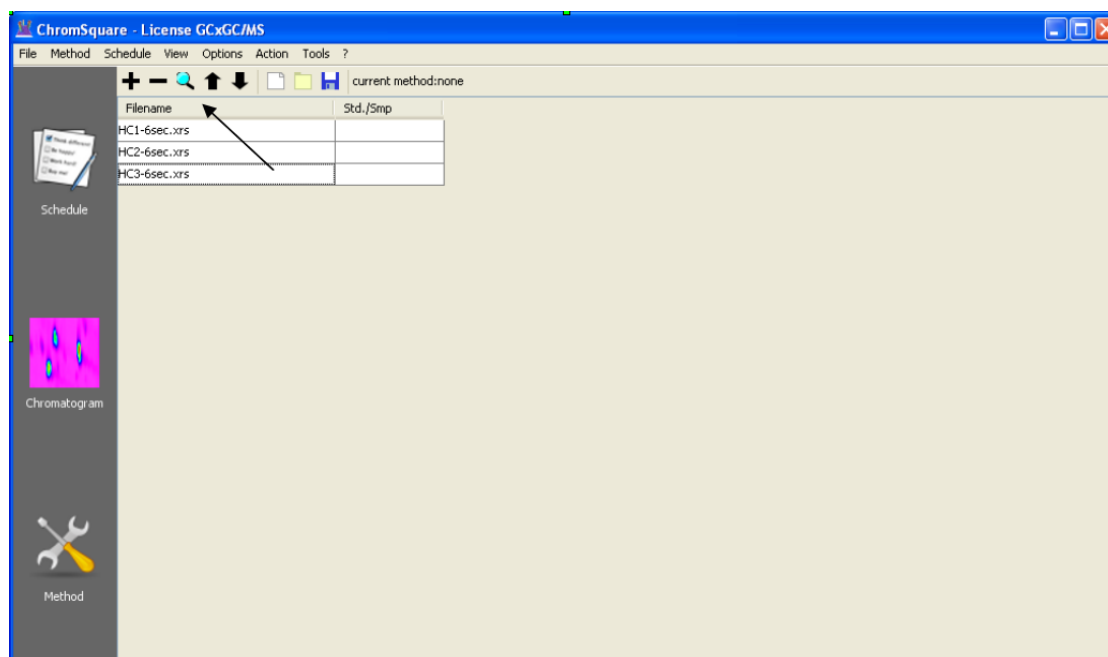


Figure 9.9: The icon for loading the selected chromatogram

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 7 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 (7.6.2).

The Menu Action command is shown in Figure 9.10.

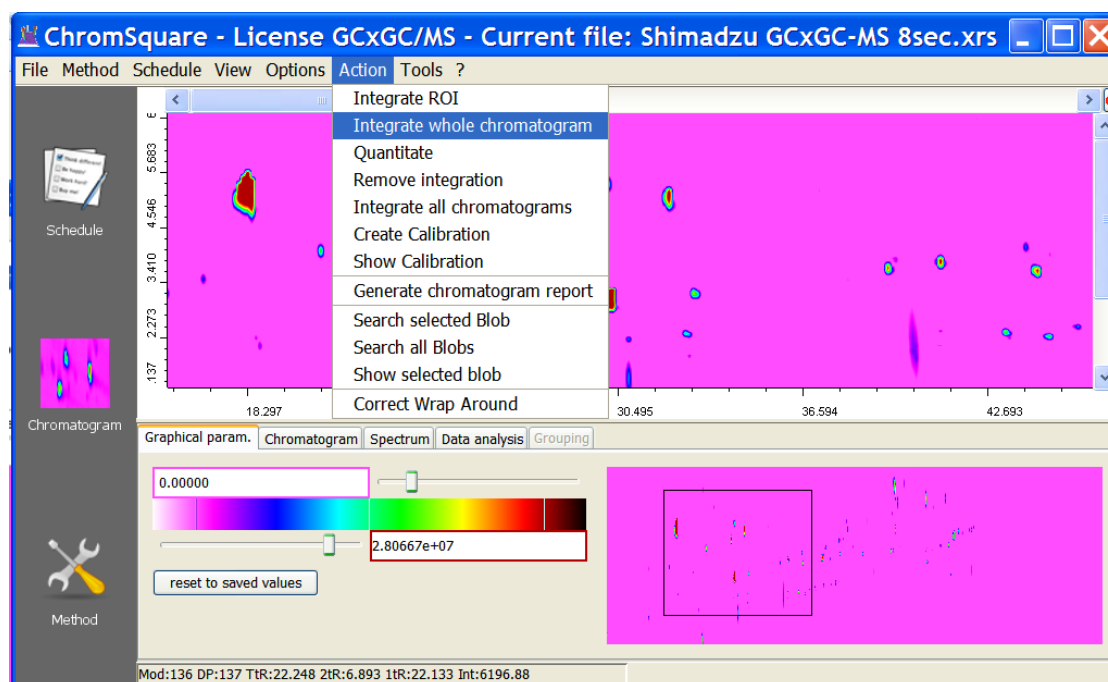


Figure 9.10: The Menu Action - Integrate Whole Chromatogram

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

The Calculation progress window is shown in Figure 9.11.

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,

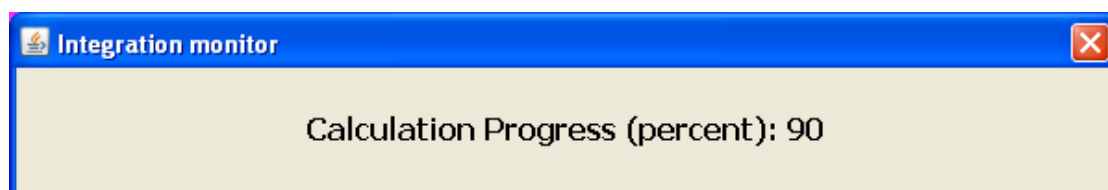


Figure 9.11: The window showing the calculation progress

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.

The Figure 9.12 shows a zoomed region of the chromatogram after the completion of the integration.

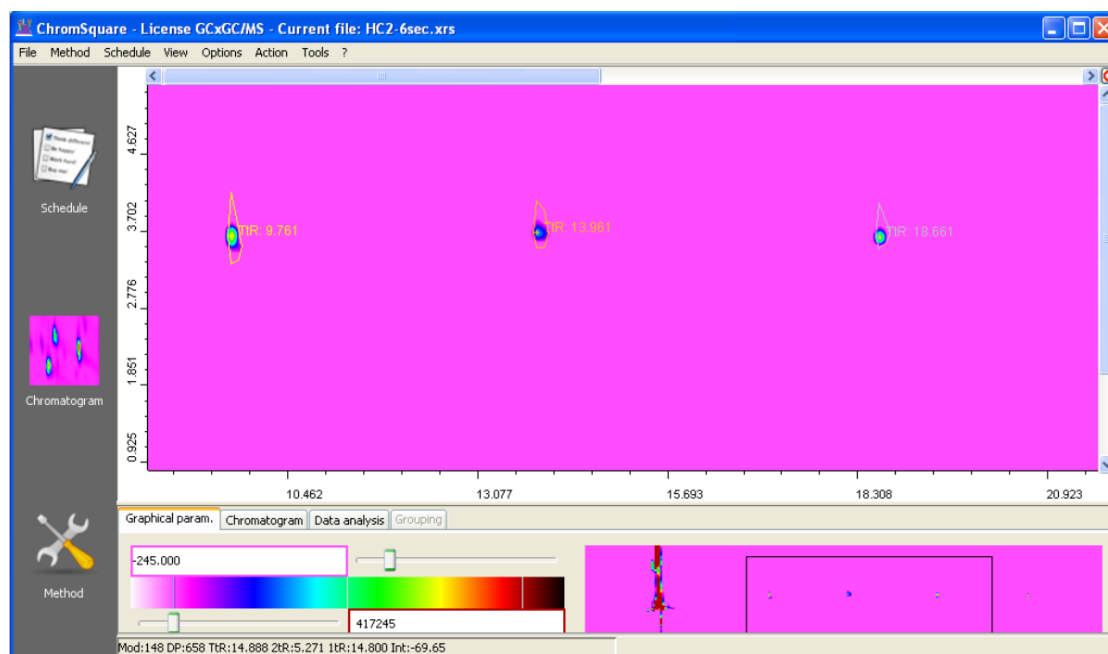


Figure 9.12: Integrated chromatogram - Zoomed view

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.

The Figure 9.13 shows a the context menu and the dialog box for the manual identification of a Blob.

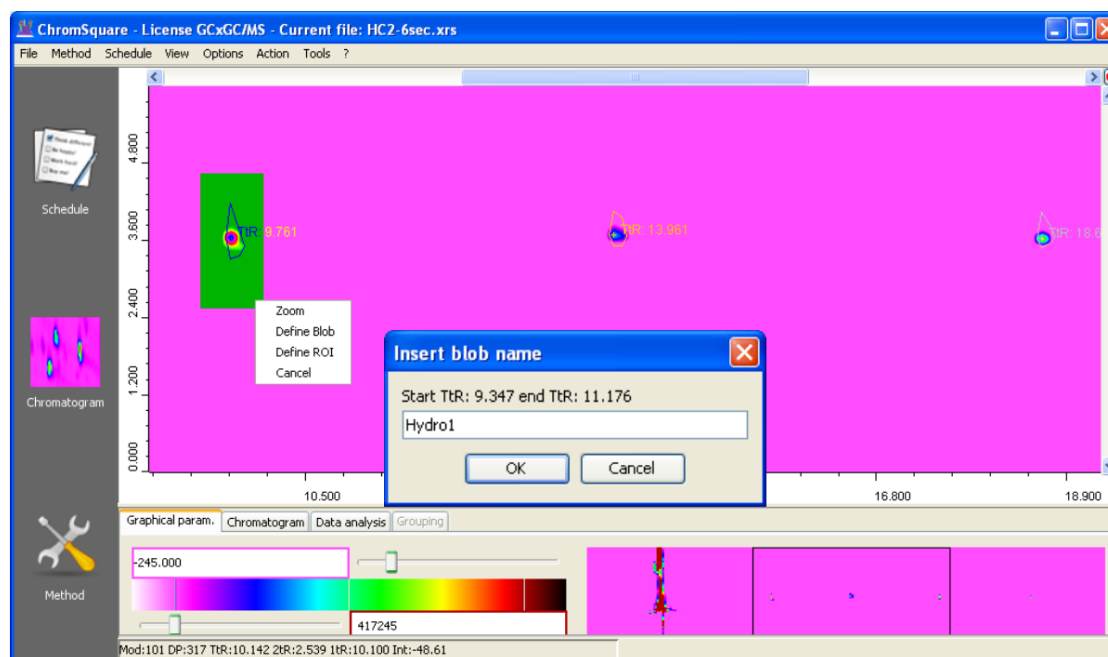


Figure 9.13: Manual identification of a Blob

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.

The Figure 9.14 shows the Method Panel with the Blob names and IDs; it also shows the Method path name.

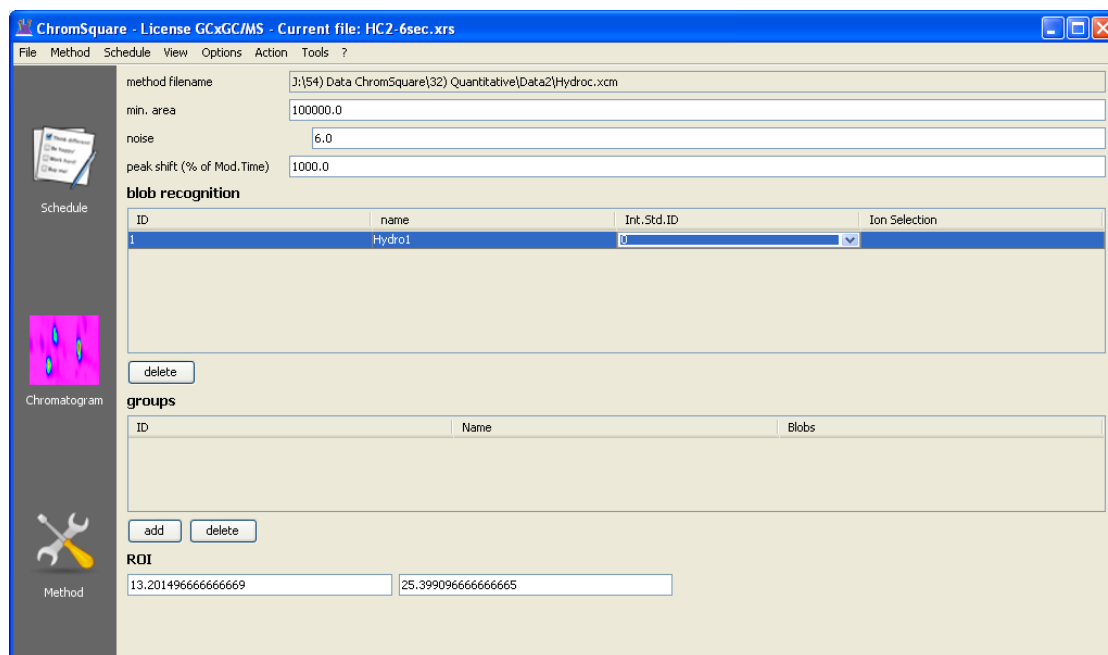


Figure 9.14: Method Panel showing Blob names and Method file name

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.

The Figure 9.15 shows the Chromatogram Panel with the Blob names (both in the Map Plot and in the Data Analysis window).

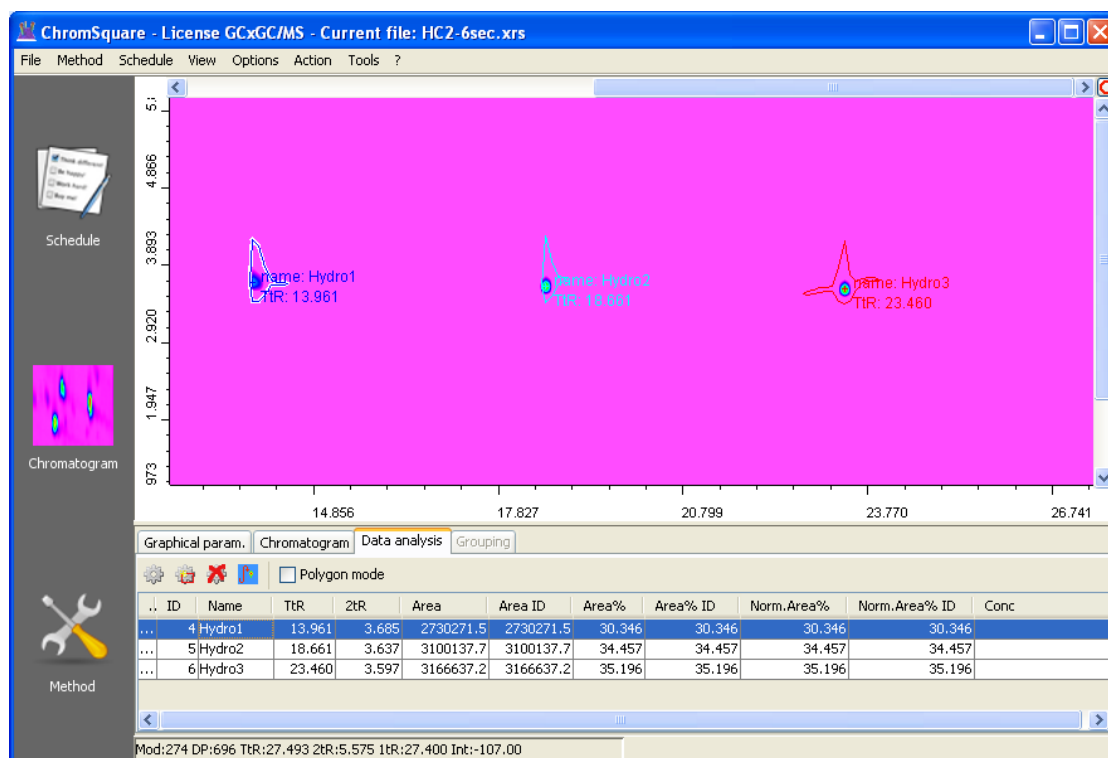


Figure 9.15: Integrated chromatogram with names of the recognized Blobs

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*.

The Figure 9.16 shows the Schedule Panel with the new columns corresponding to the Blobs.

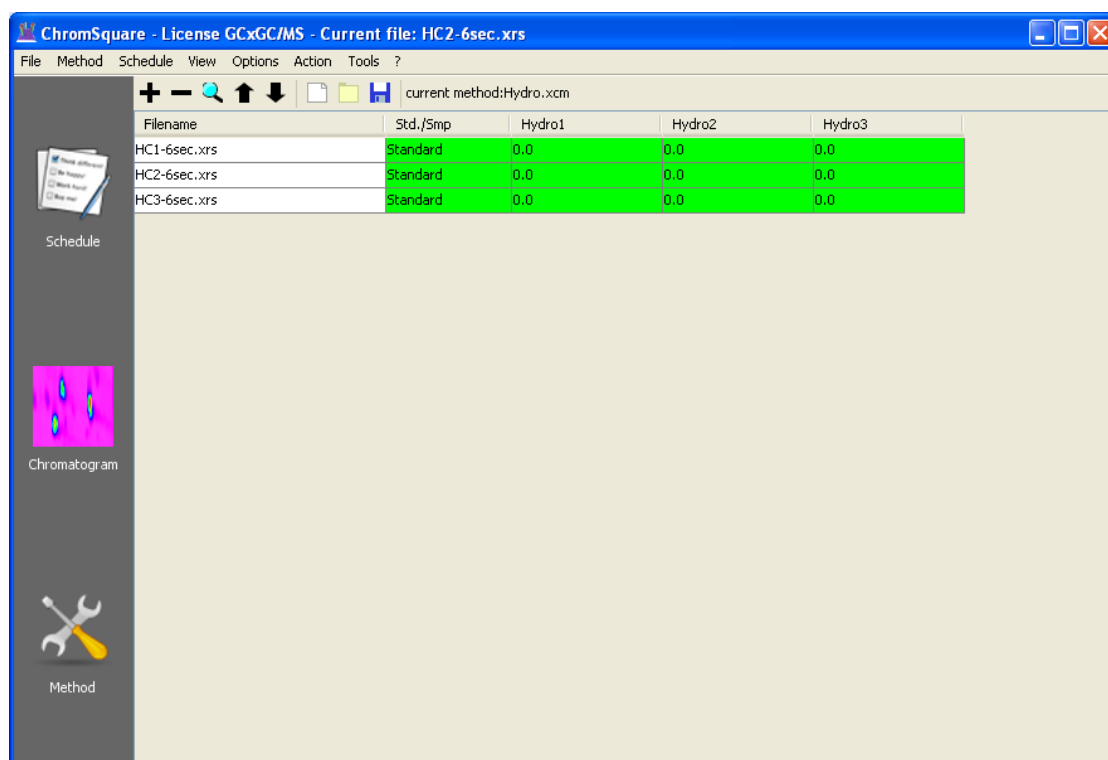


Figure 9.16: Schedule panel ready to insert concentration values

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.

The Figure 9.17 shows the Schedule Panel while a concentration value is being inserted.

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*.

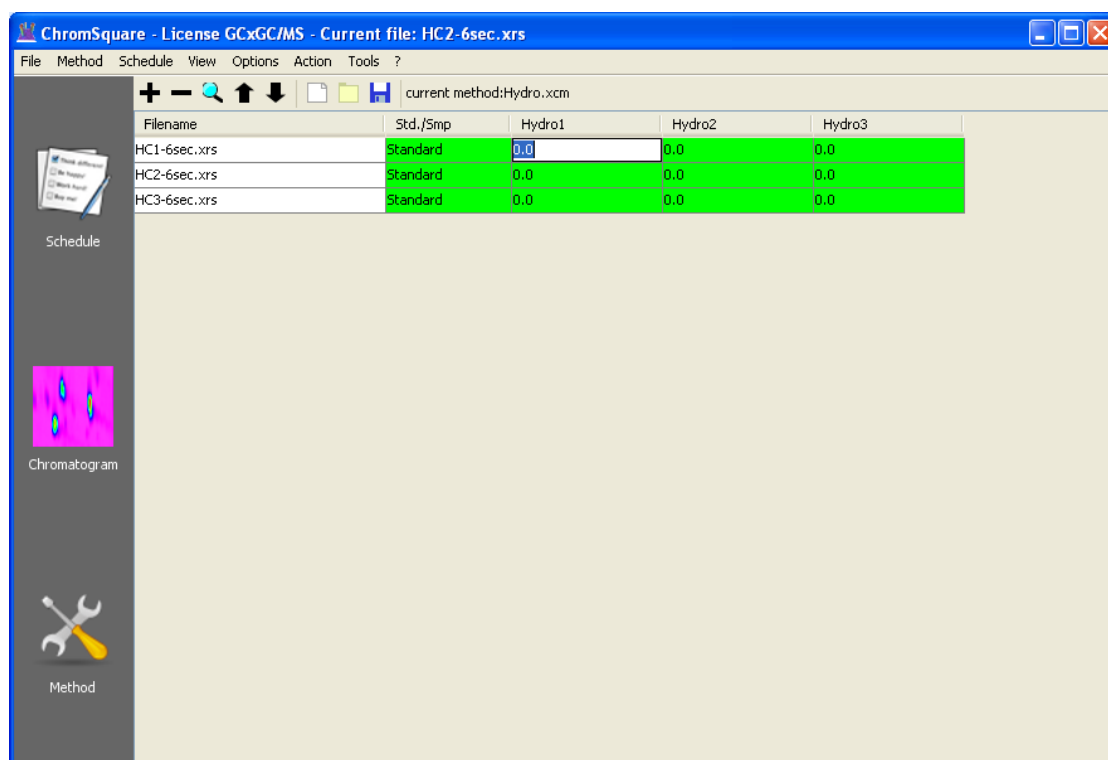


Figure 9.17: Schedule panel while inserting a concentration value

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.

The Figure 9.18 is an example of the Dialog Box which shows the calculation Results.

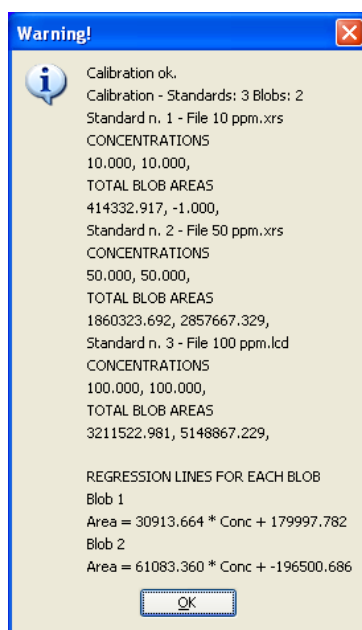


Figure 9.18: The Calibration Results Dialog box

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 9.19) 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*.

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).

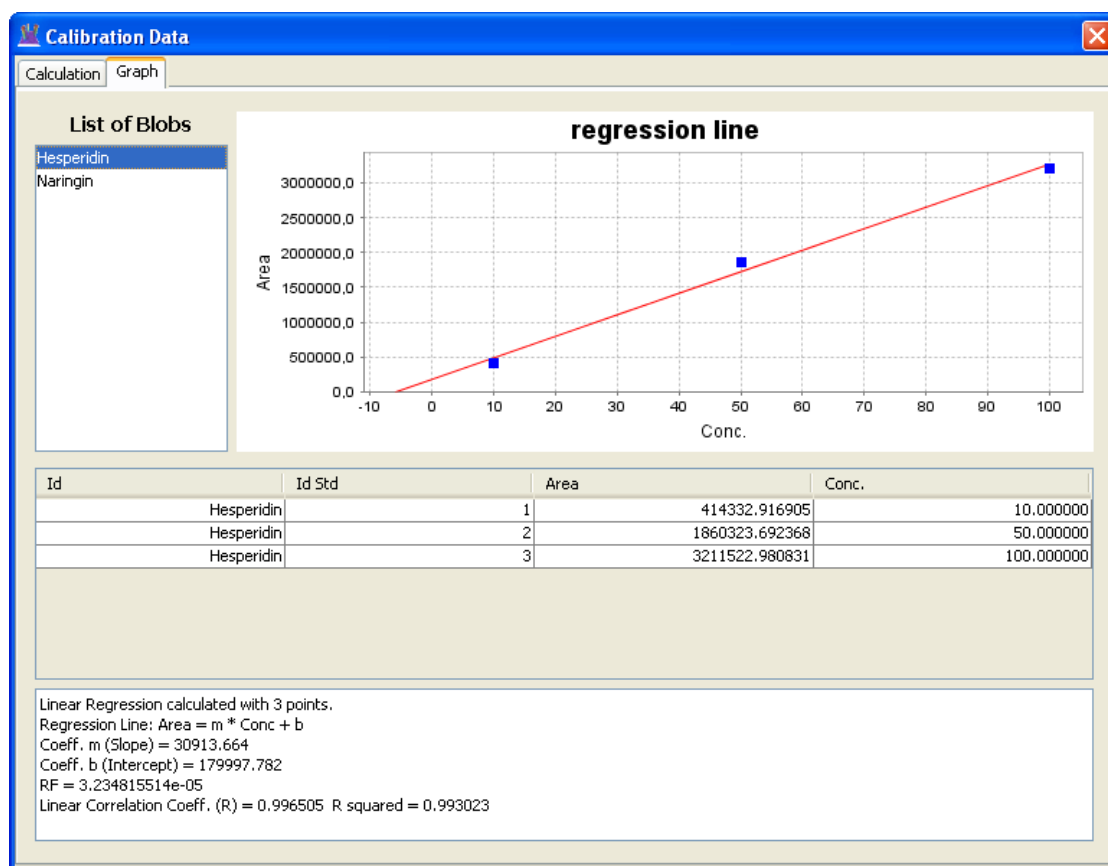


Figure 9.19: The Calibration Graph window

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 (7.6.1) in the Data Analysis window of the lower panel: the Concentration column will now be filled with the calculated concentration values.

The Figure 9.20 is an example of the Schedule Panel which shows the Quantitation Results in the yellow row corresponding to the sample.

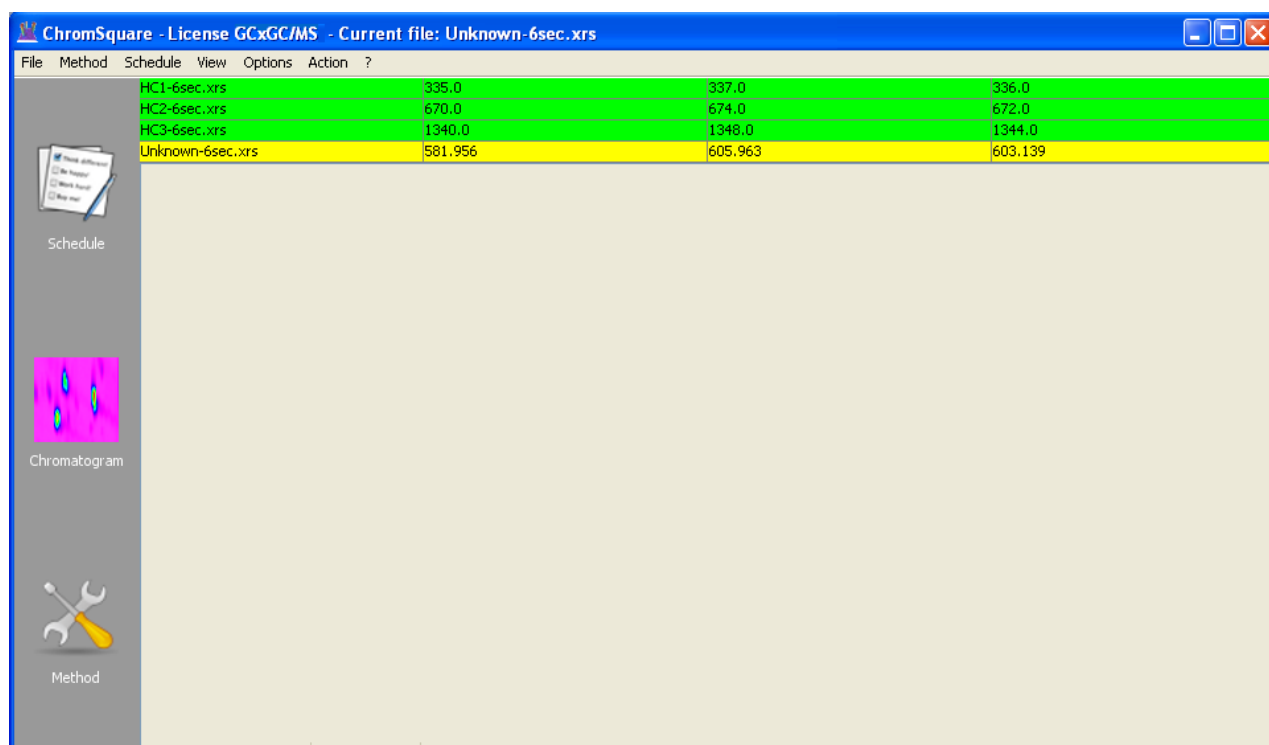


Figure 9.20: The Quantitation Results reported on the Schedule Panel

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 Cal-

ibration), 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.

A sample of Method Panel with Blob IDs and no Group is shown in Figure 9.21.

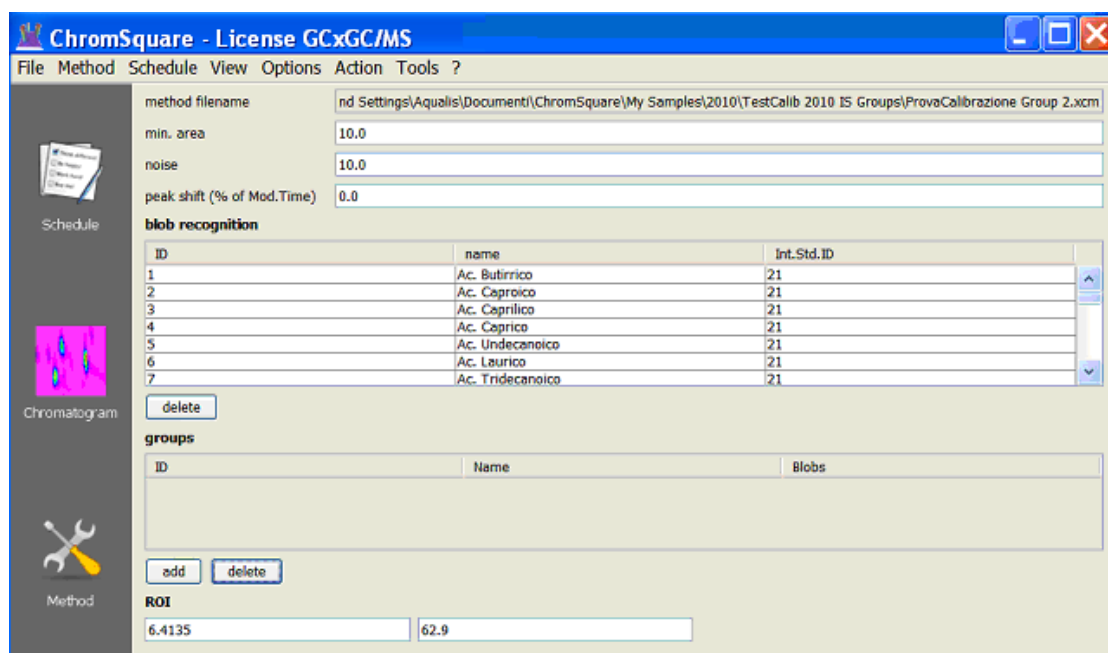


Figure 9.21: Method Panel with some Blob ID defined

- 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).

A sample of the list is shown in Figure 9.22.

- 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.

A sample of the list with selection is shown in Figure 9.23.

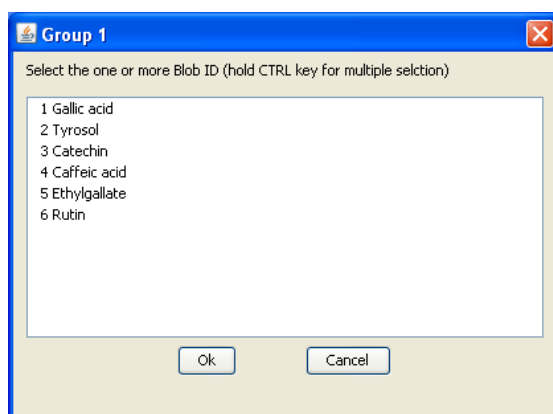


Figure 9.22: List of all Blob IDs

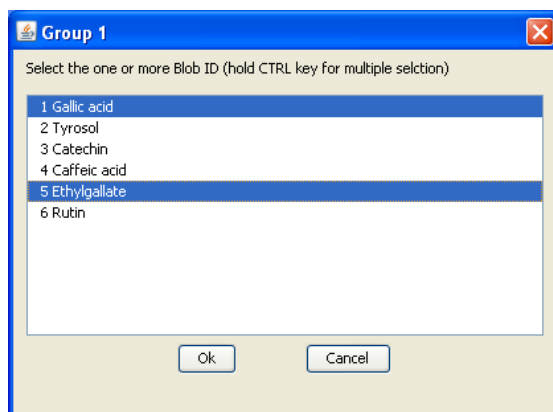


Figure 9.23: Selection of the Blob IDs

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.

See Figure 9.24 for a sample of the Method Panel with just one Group added.

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.

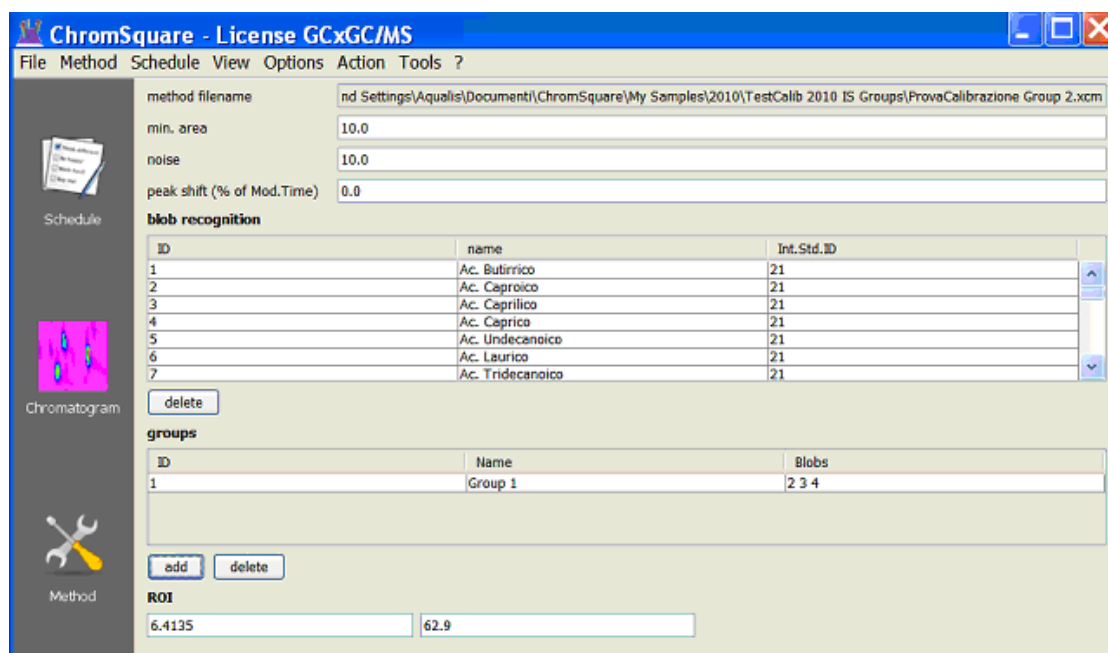


Figure 9.24: Method Panel with one Group

- It is possible to change the name of the Group (assigning a more significant term) by directly writing inside the cell table.
- The operation can be repeated for more Groups.
A more complete example is shown in Figure 9.25, where 3 groups have been defined and renamed.
- 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.
- 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.

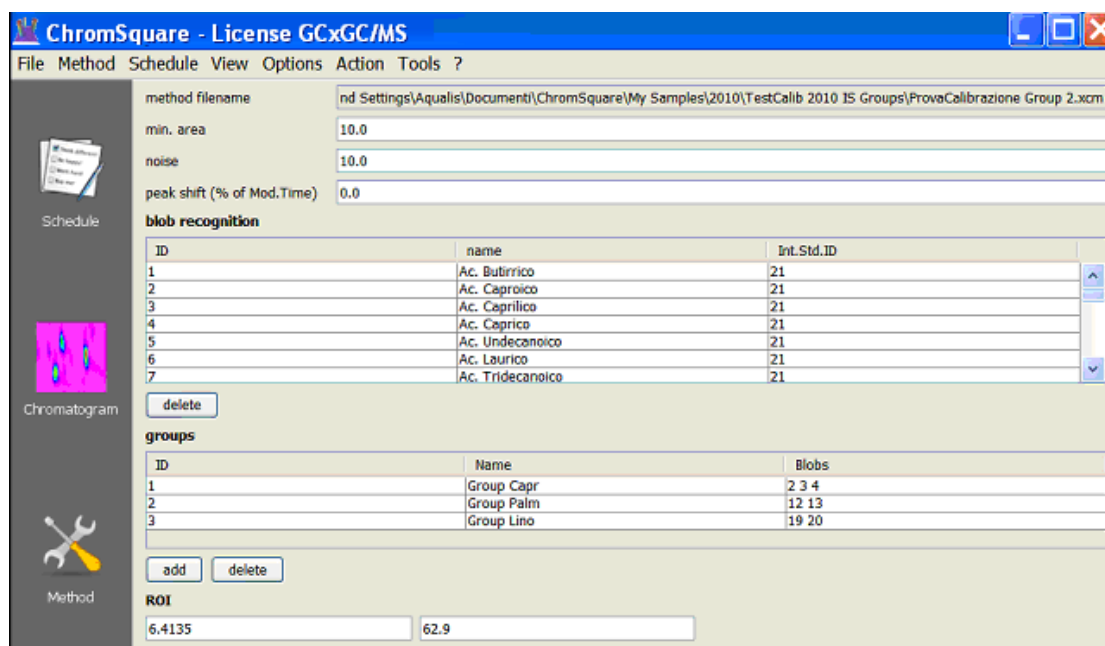


Figure 9.25: Method Panel with more Groups

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:

A sample of the Data Analysis table is shown in Figure 9.26.

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.

A sample of the Grouping Results table is shown in Figure 9.27.

This table reports a row for each Group defined in the Method, and 5 columns:

²Note 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.

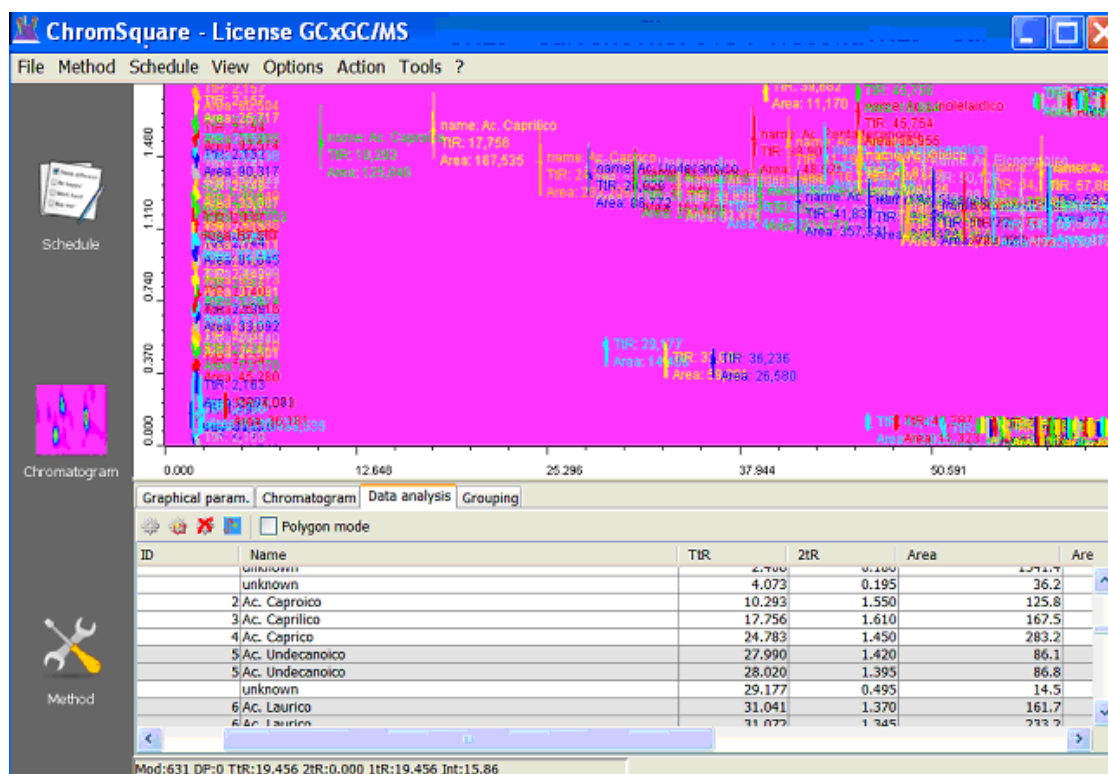


Figure 9.26: Integration Results with Named Groups

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).

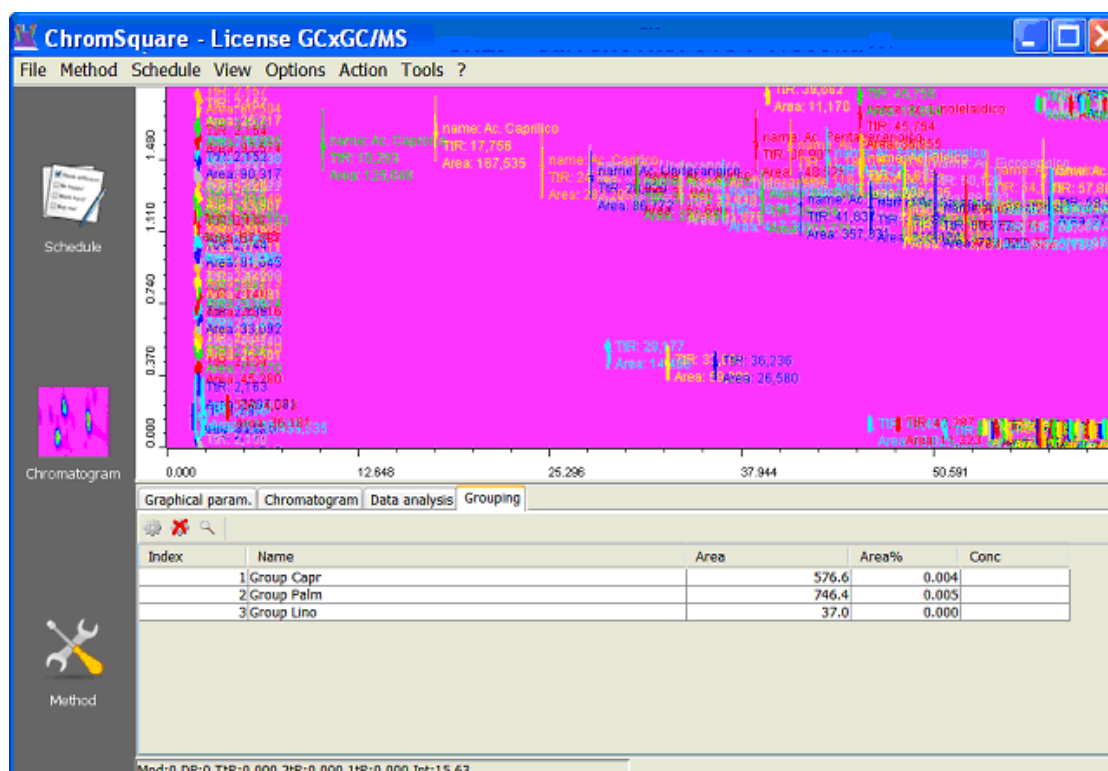


Figure 9.27: Integration Results: Grouping Results Table

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 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.

A sample is shown in Figure 9.28.

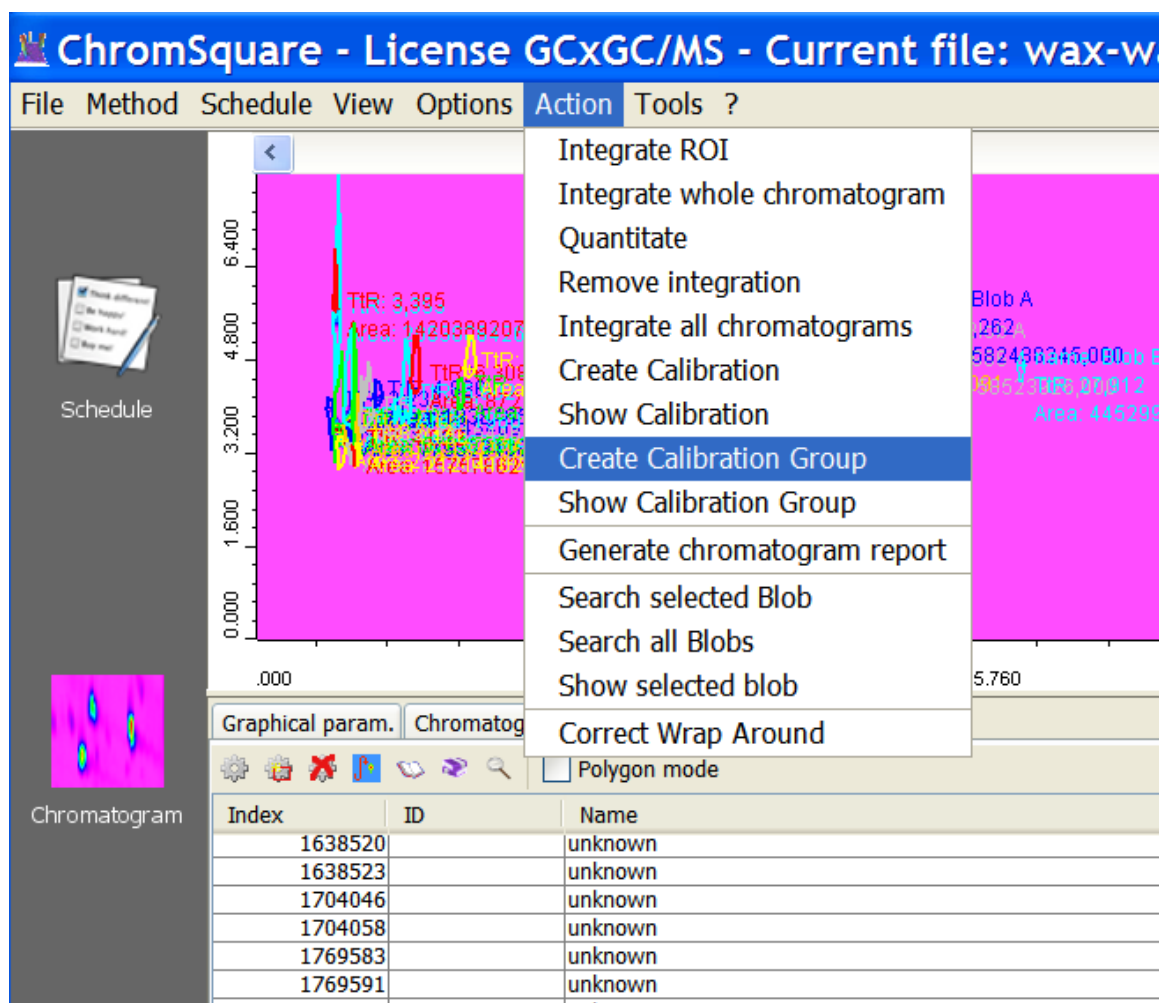


Figure 9.28: Group Calibration Command

2. An *informative window* with a summary of the calculations will be shown; give Ok to close the window.

A sample of the informative window is shown in Figure 9.29.

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.

A sample of the Calculation page is shown in Figure 9.30.

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

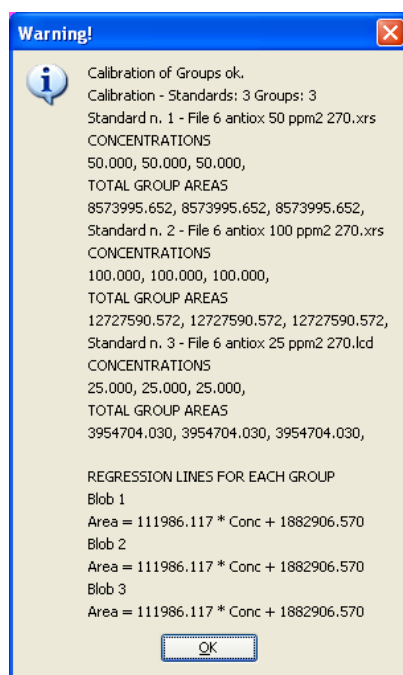


Figure 9.29: Group Calibration Summary

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.

A sample of the Graphs page is shown in Figure 9.31.

4. The command **Action|Show Calibration Group** allows the user to view the former windows without perform again all calculations.

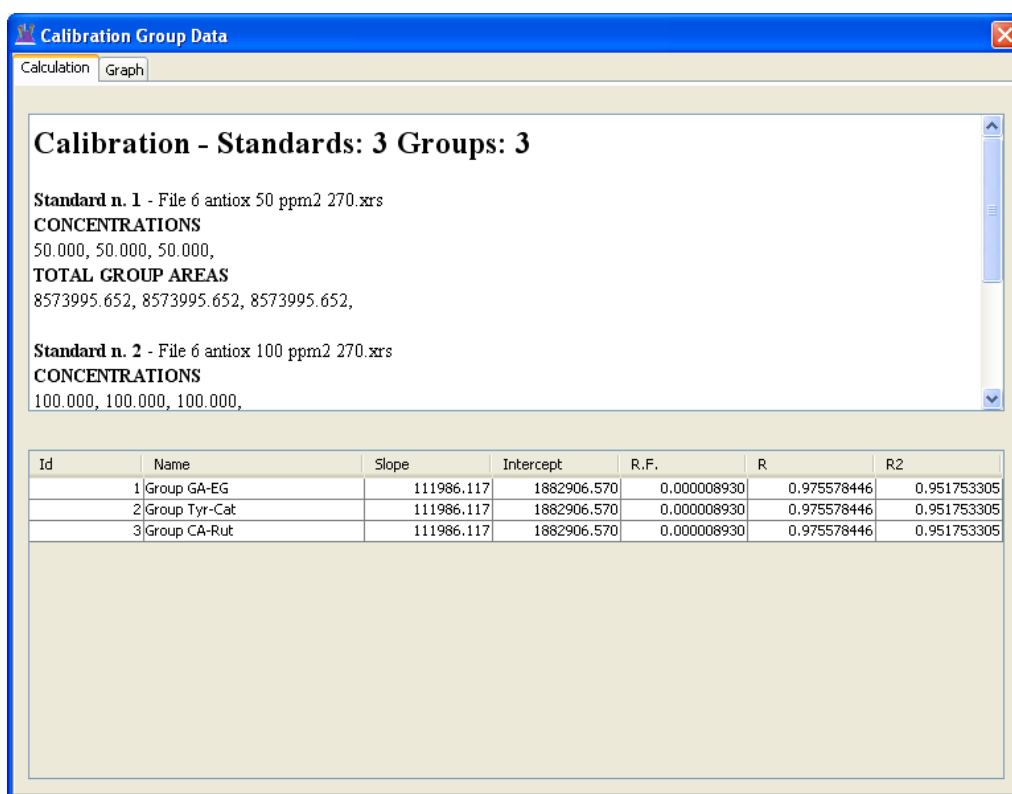


Figure 9.30: Group Calibration Data

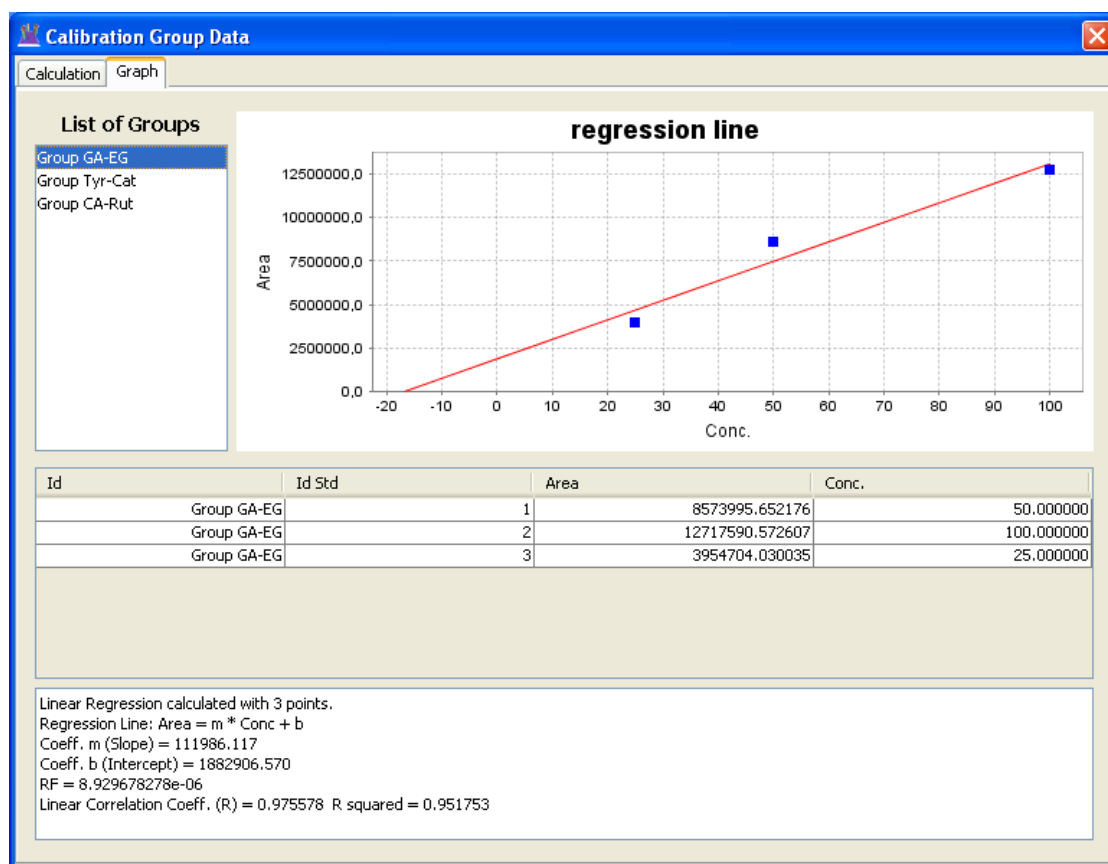


Figure 9.31: Group Calibration Graph

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.

The next two figures show the Data Analysis page and the Grouping page with the Quantitation results of a given sample.

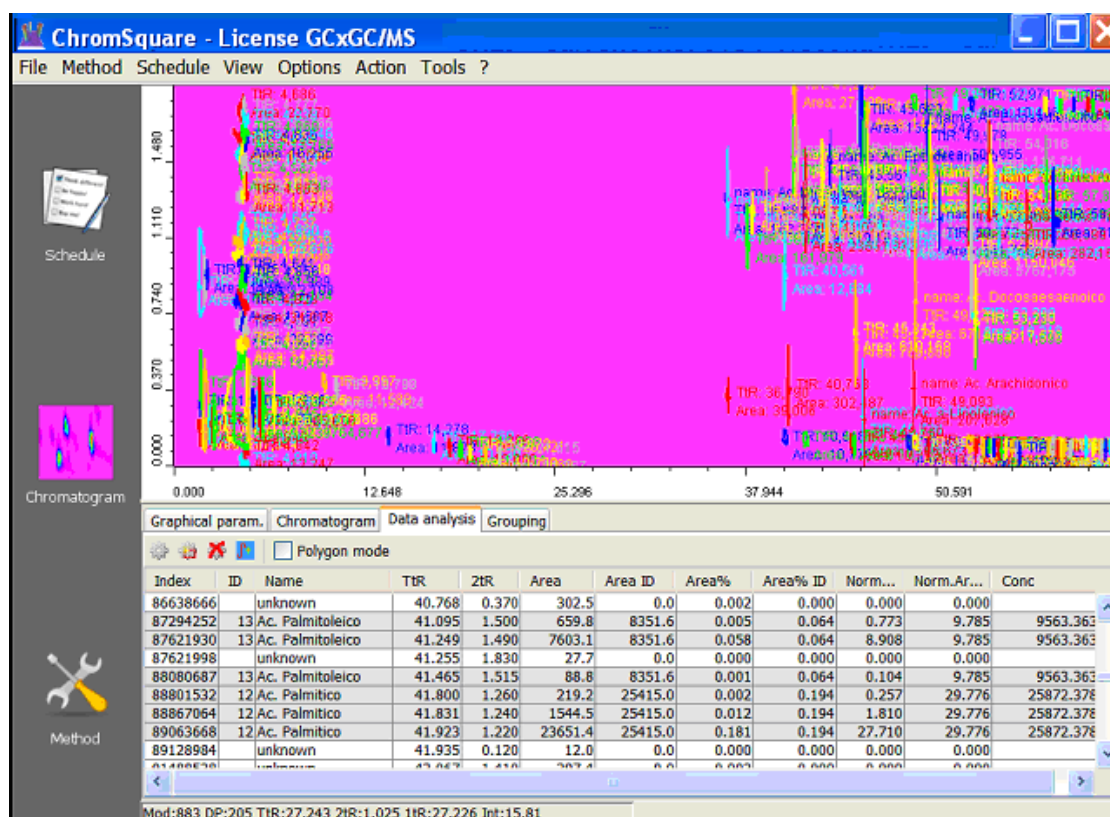


Figure 9.32: Group Calibration - Quantitation Results - Data Analysis Page

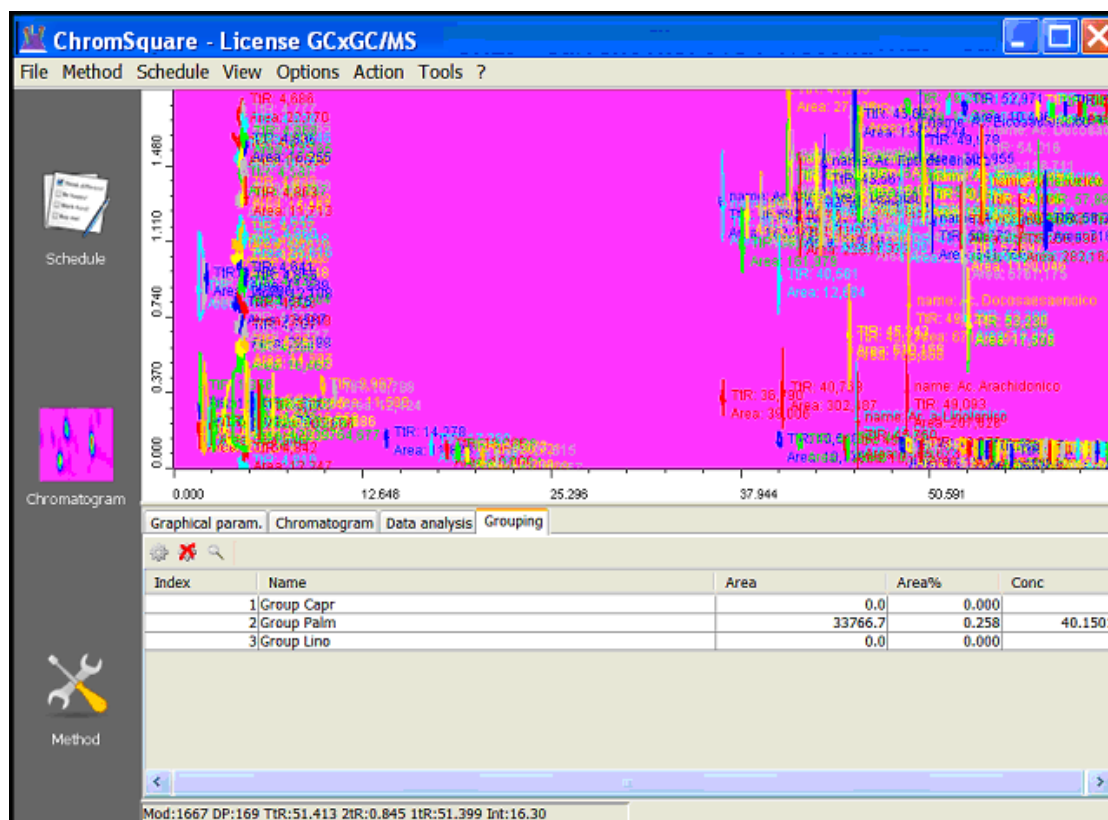


Figure 9.33: Group Calibration – Quantitation Results – Grouping Page

Chapter 10

Advanced Topics

10.1 3D View

10.1.1 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 7.2.2 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 10.1.

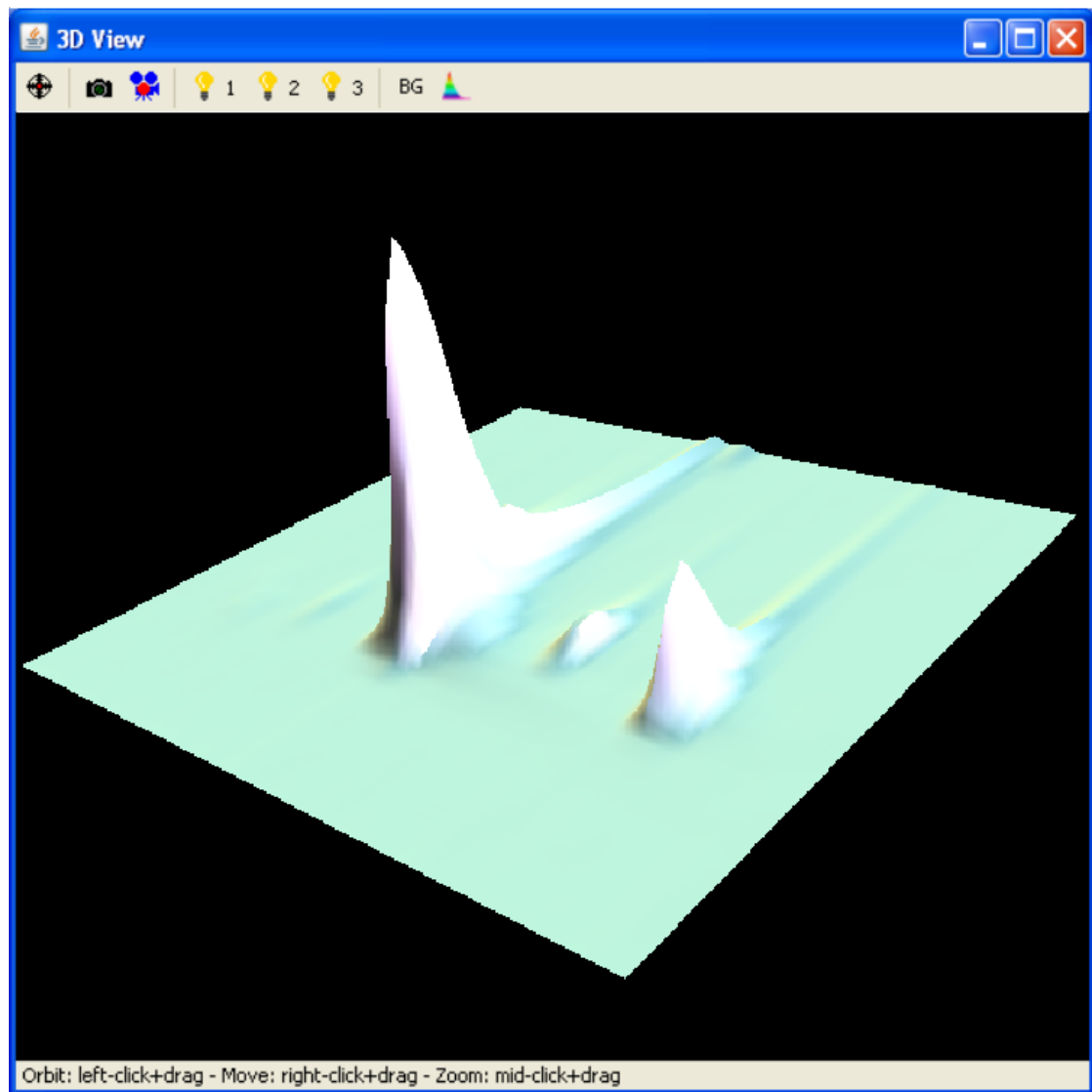


Figure 10.1: 3D View Window - Initial View

10.1.2 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.






10.1.3 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 Box is 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.

¹For 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 10.2.
- **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).

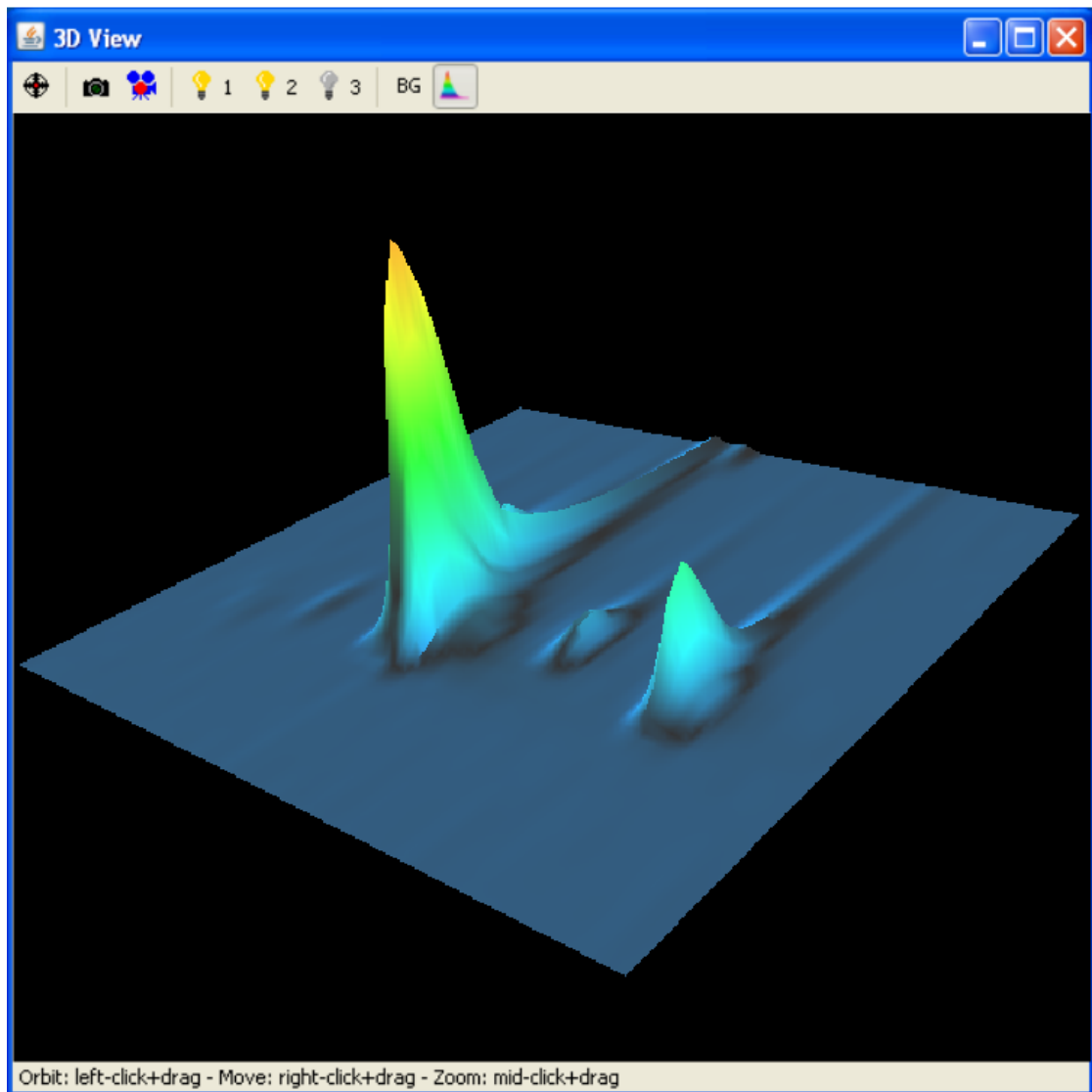


Figure 10.2: 3D View Window - Surface colorization

10.2 Compare/subtract tool

10.2.1 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.

10.2.2 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.

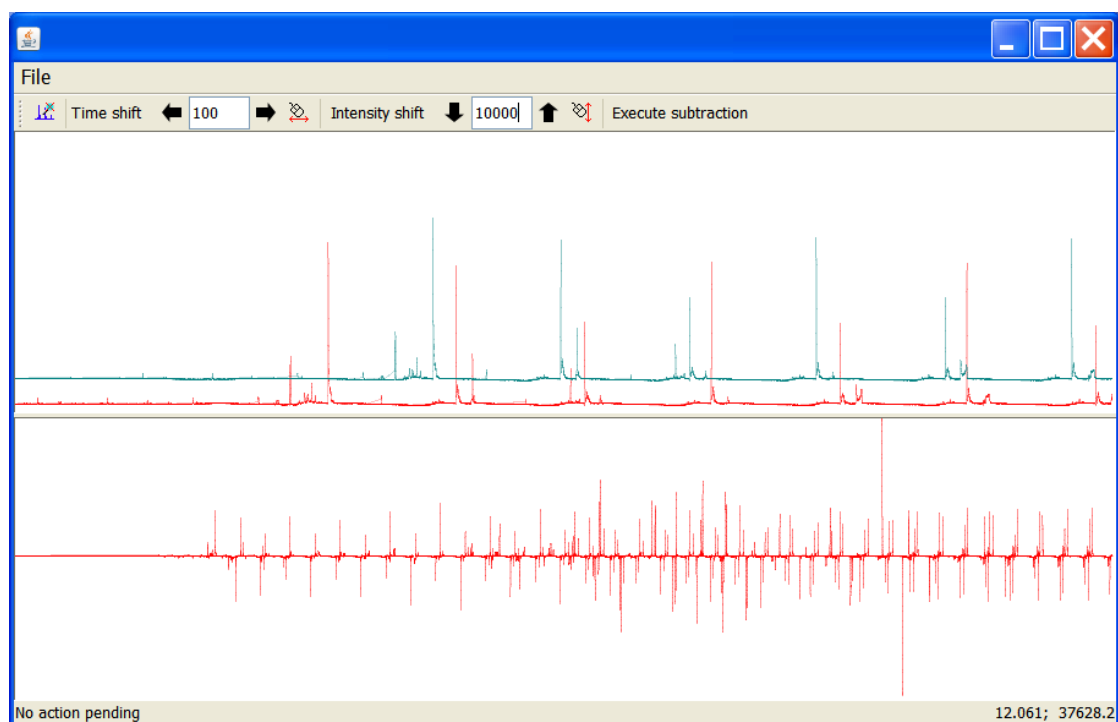


Figure 10.3: Compare/Subtract Tool

10.2.3 Description of the Compare/Subtract window

The Compare/Subtract window (shown in Figure 10.3) contains:

- a File menu;
- a Tool Bar with various icons and controls, shown in Figure 10.4;
- 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.

10.2.3.1 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 7.2.3 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 (10.2.3.3) 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.

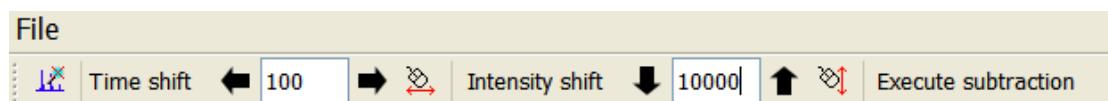


Figure 10.4: The Compare/Subtract Window Tool Bar

10.2.3.2 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 Box for the choice of an existing chromatogram, which will become the *Base Chromatogram*.
- **Open Reference Chromatogram.** The command opens a File Selection Dialog Box for the choice of an existing chromatogram, which will become the *Reference Chromatogram*.
- **Save Result Chromatogram As.** The command opens a File Selection Dialog Box for 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.

10.2.3.3 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 fields.



The command allows the user to shift the reference chromatogram along the *time* 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.

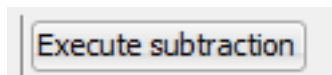


The command shifts the reference chromatogram *up* along the intensity axis by an amount indicated in the text editor.



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.

10.2.3.4 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.

10.2.4 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 (10.2.3.3) or mouse commands (10.2.3.4).
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**.

10.3 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 10.5 shows a sample of a polygonal region being defined.

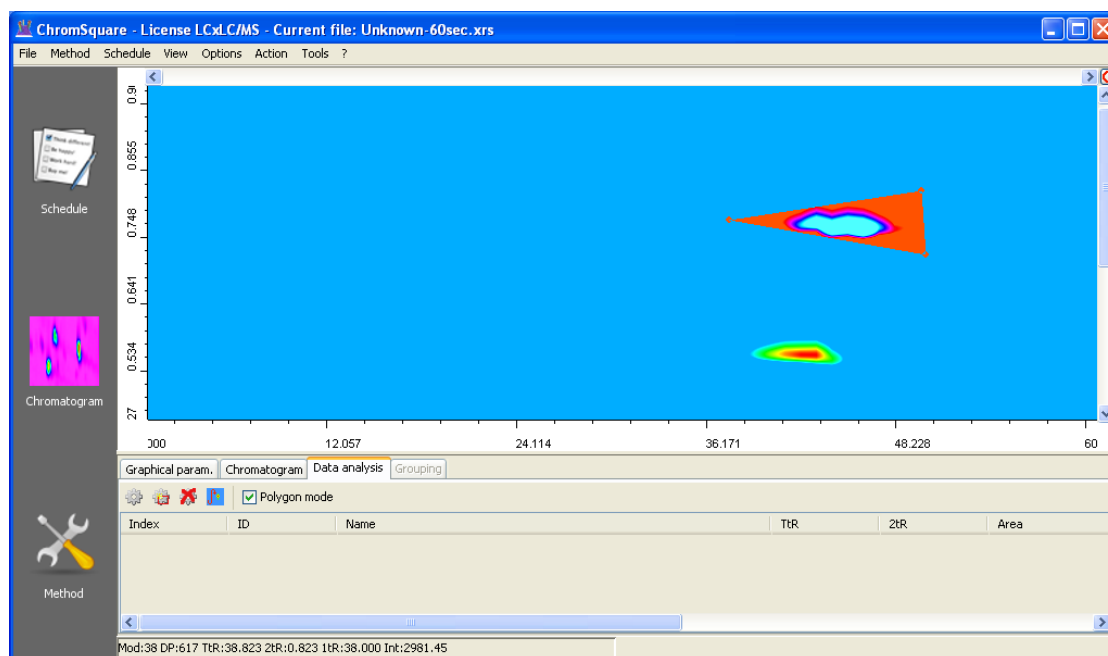


Figure 10.5: Defining a Polygonal Region

Figure 10.6 shows the assignment of the name to the defined region.

Figure 10.7 shows the integration results with the identification of the previously assigned polygonal Blob ID.

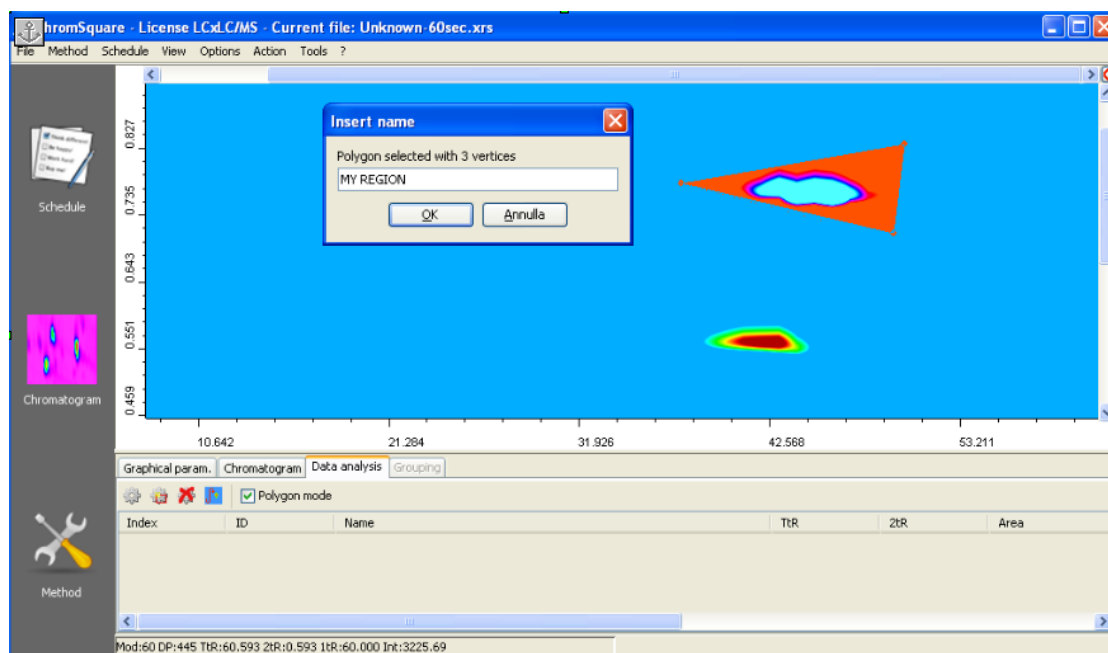


Figure 10.6: Entering the polygon region name

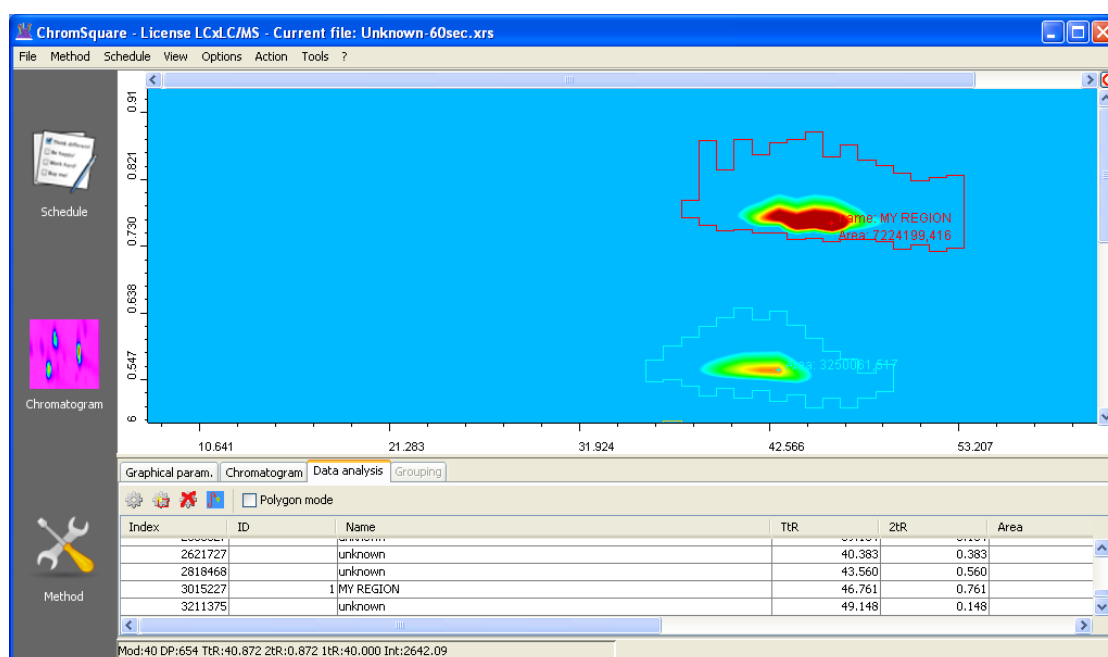


Figure 10.7: The polygonal Blob after integration

10.4 Profiles

10.4.1 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 the installation 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.

10.4.2 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 Box will 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 10.8).

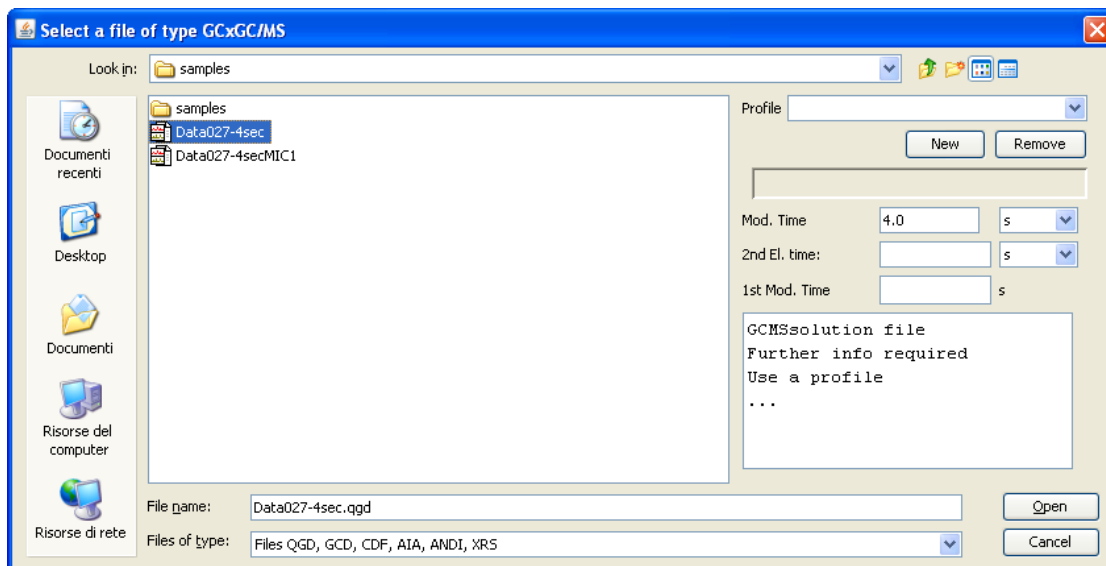


Figure 10.8: Open Dialog Box with Profile Combo Box

4. Press the **New** button; a new Dialog Box, named **New Profile**, will open. See Figure
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.

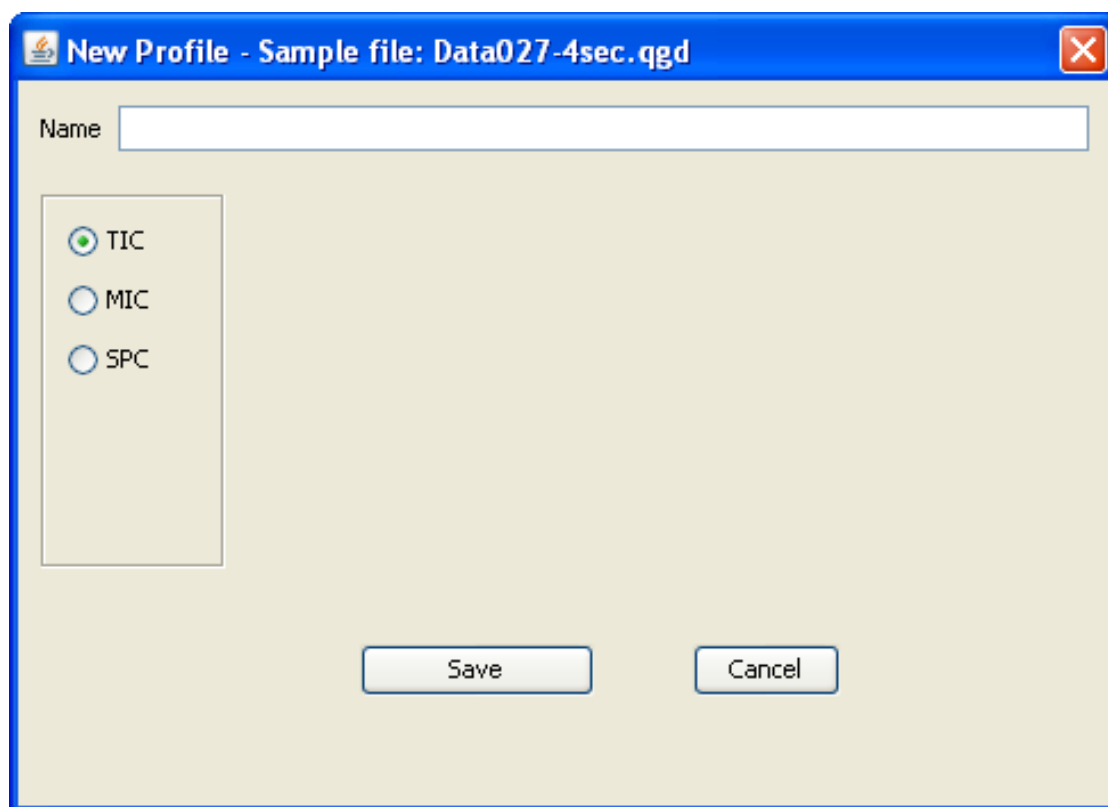


Figure 10.9: The New Profile window

10.5 Configuration

10.5.1 Description

This section describes some technical features of *Chrom^{square}* software. Its content is oriented to software specialists (System Administrators, IT people). However, some information can be useful also for common users.

10.5.2 The file `Options.xml`

This file maintains some software options between different executions; for example, when the user changes one or more visualization options (see 4.5), the information is stored in this file.

The file is a text file, coded according to the rules of the XML language.

A prototype of the options.xml is generated in the installation folder by the installation procedure. At first execution, the software creates a copy of the file in the Windows temporary folder ²; all modifications of the original options are stored on the copy of the temporary folder.

Most options are managed directly by the software, so that the user does not normally modify the file (wrong changes could make the file unreadable). Manual modifications are required for less common options.

10.6 Ion selection

10.6.1 Description

If the chromatogram under analysis is generated by a mass spectrometer, a selection of fragments can be used for quantitation instead of the whole intensity. This procedure is called *Ion Selection*.

Note: *When data file is generated using GCMSsolution, profile “SPC” has to be used during loading procedure.*

The operator can choose what are the ion(s) that have to be summed to calculate the Blob area using *Ion Selection* information stored in the Method.

Ion Selection information is specified through the last column (the 4th column) of the Blob recognition table of the Method Panel.

Once a Blob has been defined in the Method Panel, in the same row of name and topology definition, a text describing Ion Selection can be entered in the last column, as shown in Figure 10.10:

Moving the mouse around the Ion Selection column, a small yellow window (a *tooltip*) is shown, which specifies the syntax to be used to describe Ion Selection information.

There are 3 possibilities:

1. If the cell is left blank, the whole intensity is taken, and all components are summed.

²This folder varies according to the MS Windows version:
In Windows XP this is C:\Documents and Settings\user\Local\Temp\
In Windows 7 it is C:\Users\user\Local\Temp\
Where *user* stands for the current user login

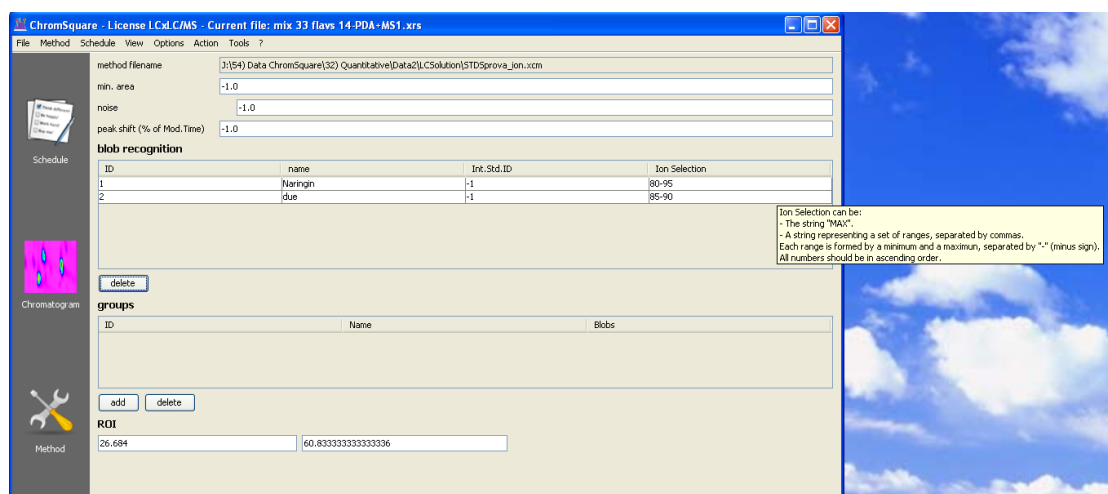


Figure 10.10: Method Panel with Ion Selection

2. The text "MAX" means that the system should take into account the most abundant fragment.
3. The user can also provide a list of ions, by means of a set of items separated by commas; each item can represent a single ion number (M/Z) or a range of ion numbers; the range is described by the starting and ending M/Z numbers separated by dashes. All numbers should be in ascending order.

For example, the following text:

60-80, 96, 99, 105-107

means that all ions between 60 and 80, those between 105-107, plus ion 96 and 99 concur in determining the Blob area.

The results are computed upon those values.

10.7 Assembling Data Files

10.7.1 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).

10.7.2 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 10.11.

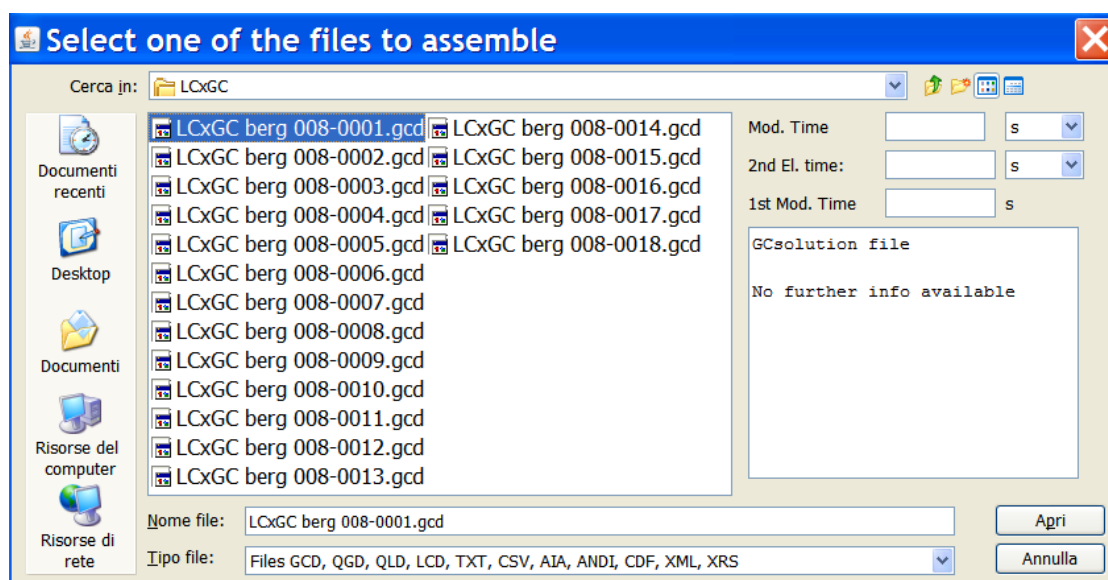


Figure 10.11: The File selection Dialog box of the Assemble case

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 10.12.
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 10.13.

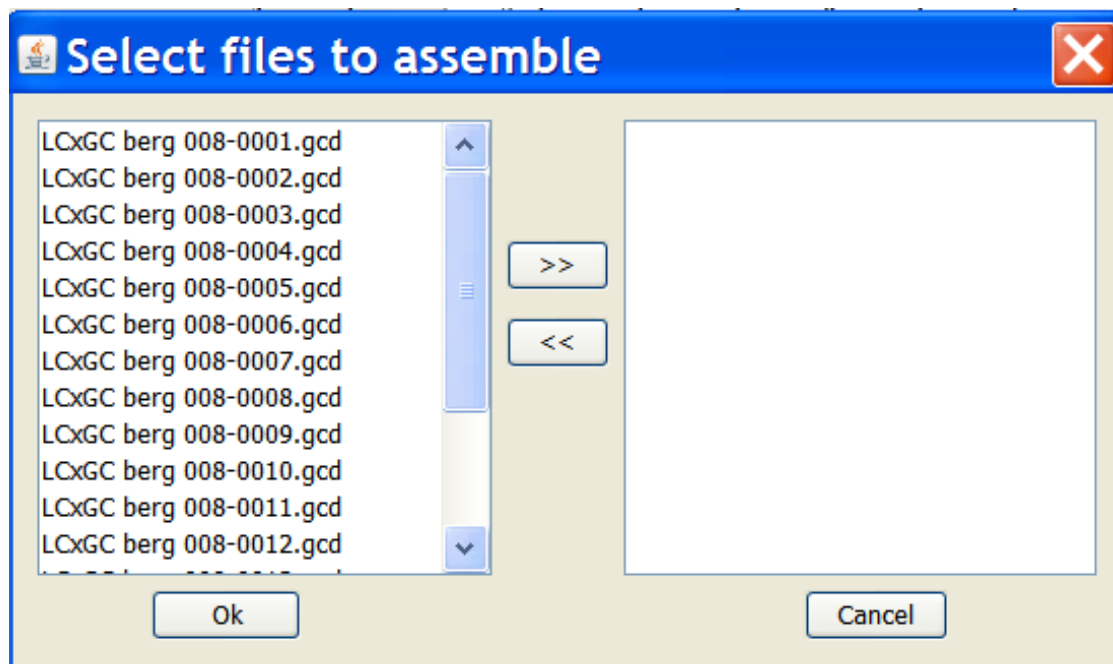


Figure 10.12: List of the files to assemble

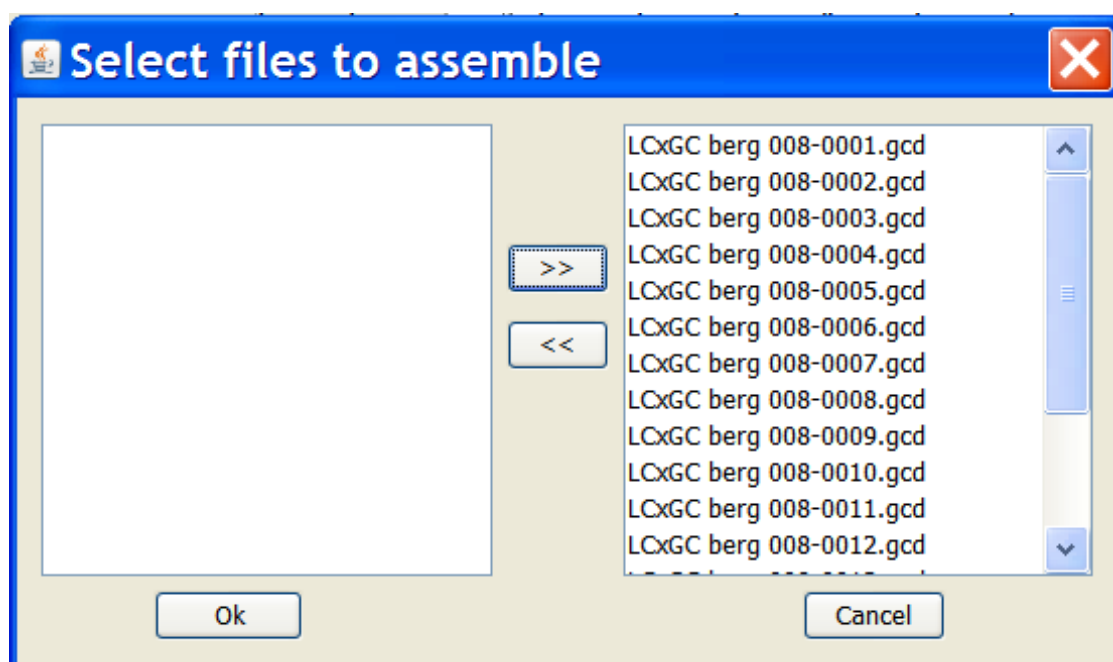


Figure 10.13: Files selected for assembling

5. Press **Ok** when the desired configuration is achieved. A new dialog box is finally shown, asking for confirmation (see Figure 10.14).

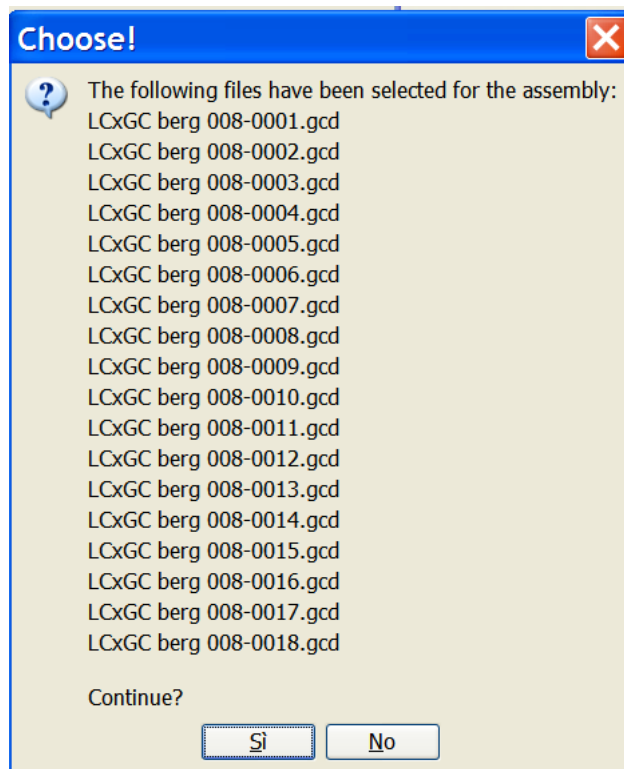


Figure 10.14: Asking for confirmation before assembling

6. The software will load all single datafiles, assembling them into an unique *Chrom^{square}* datafile “.xrs”, that will be saved in the same directory. 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).
7. At the end of the procedure, the resulting assembled chromatogram is displayed (see Figure 10.15)

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 than in the other cases: this could cause some limitations at zoom level.

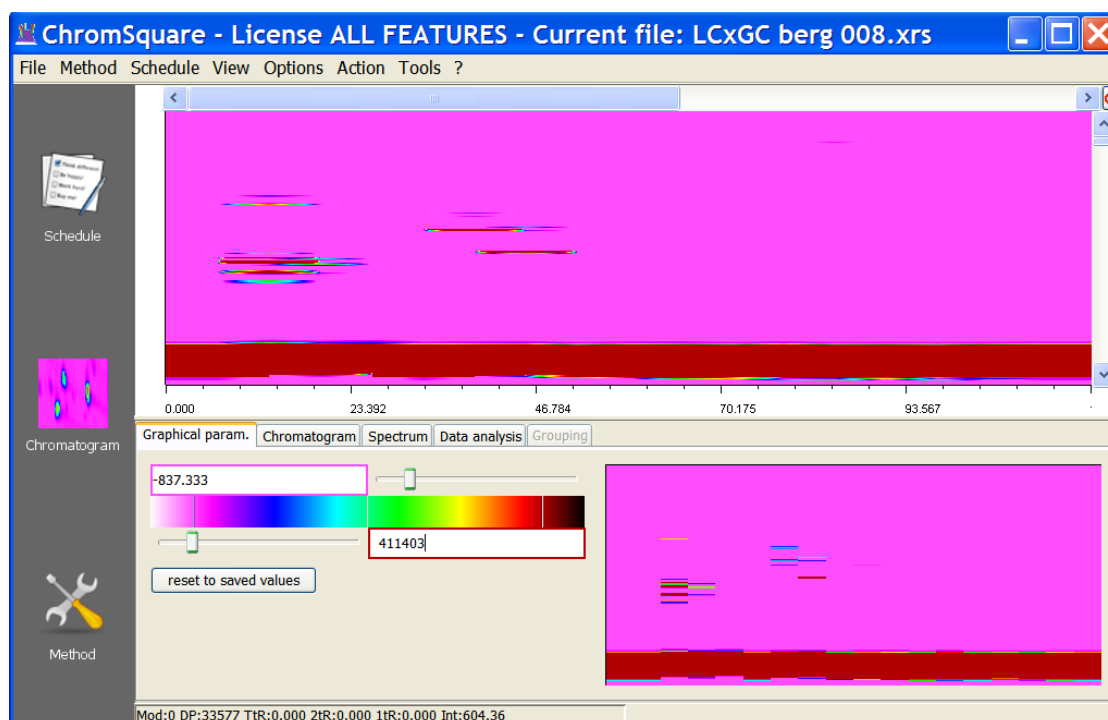


Figure 10.15: The resulting assembled chromatogram

Chapter 11

Technical Notes

11.1 Prerequisites to install ChromSquare with GCMSsolution spectra search

11.1.1 Description

Since version 1.5 the spectra search function is deeply integrated with GCMSsolution.

An agreement with Shimadzu Co. allowed to perform a seamless search, but a version of GCMSsolution higher than 2.71 is required.

The steps to upgrade are:

- Install GCMSsolution 2.71 according to the instructions provided by Shimadzu.
- Run ShimadzuDllSetup.exe to enable the communication API.
- Install ChromSquare

The spectra search function will not work with previous versions of GCMSsolution.