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# Installing and starting Chrombox Q

## Windows computers:

- Download the installation and unzip the archive “[qq.zip](#)”
- Move folder “**QQ**” to the preferred destination, e.g. [ [C:\Chrombox\](#) ], This will be the Q-root folder
- If Installed on a local disk or on a memory stick Chrombox Q can usually be started by using the “Chrombox Q.exe” file in the Q-root folder.
- If installed on a network disk you may have to use one of the methods described below:
- Find the file “[qstart.m](#)” in the folder “[...\qq\various](#)” and move it to somewhere in your Matlab path. This is the only file that needs to be in the Matlab path. Possible destinations may be found by starting Matlab and typing “[path](#)”.
- Open the “[qstart.m](#)” and edit the run command to it points to the file “[qq\\_startscript](#)” (see highlighted line below).
- An example of “qstart.m” is shown below. The part to edit is shown in blue.

---

```
% Startupscript for Chrombox Q
% Starts startscript by the run command.
% Startscript must be located in the Q root,
% qstart must be in the matlab searchpath
% run c:\qq\qq_startscript
run c:\CHROMBOX\QQ\qq\_startscript
```

---

- You can also create a desktop shortcut by copying the shortcut to Matlab and adding the following to the destination “[/automation /r qstart](#)” An example of how it can look is shown below, the added part in blue:

```
C:\MATLAB6p5\bin\win32\matlab.exe /automation /r qstart
```

## Mac computers (OS X):

- Download the installation and unzip the archive “[qq.zip](#)”
- Move folder “**QQ**” to the preferred destination, for example [ [/Users/yourname/Documents/CHROMBOX/qq](#) ], This will be the Q-root folder
- The shell script [macstart\\_q.command](#) stored in the Q-root folder can be used to start the program if the file is executable and Matlab can be started with the terminal command “matlab”. Note that the extension “.command” may be hidden in Finder.



- To check if Matlab can be executed by “matlab” open the terminal and type “matlab”. If Matlab does not start you can do the following:
  - Put a symbolic link to Matlab in your path by opening the terminal and typing “sudo ln -s /Applications/MATLAB\_RXXXXX.app/bin/matlab /usr/local/bin” where RXXXXX should be replaced by the Matlab version number, for example “R2017a”. Alternatively, open “Applications” in Finder. Locate Matlab, right-click and select “Show Package Contents”. Open the folder “bin” and locate the application file “matlab”. In terminal type “sudo ln -s ” without pressing enter. Thereafter drag the “matlab” application file to the terminal. Ensure there is a space between “-s” and “/Applications” and press enter.
- To make macstart\_q.command executable, do the following:
  - Open the terminal. Use “cd” to change directory to the Q root where the macstart\_q.command is located or open the terminal at the Q root folder if that is an option. Type “chmod +x macstart\_q.command”. Alternatively, type “chmod +x ” without pressing enter and drag the macstart\_q.command file from Finder to the terminal. Ensure there is a space between “+x” and “macstart\_q.command” and press enter.
- Thereafter double-click on macstart\_q.command in Finder to start the program. Depending on your security settings you may get the following message: “macstart\_q.command” can’t be opened because it is from an unidentified developer. To solve this, open System Preferences – Security and Privacy – General and press [Open anyway] next to the message regarding the file. An alternative way of allowing the file to be executed is to open the file in TextEdit and saving it again. Then it will no longer have status as downloaded from the Internet.

As an alternative to the above procedure, Chrombox Q can be started by the following method:

- Find the file “qstart.m” in the folder “.../qq/various” and move it to somewhere in your Matlab path. Possible destinations may be found by starting Matlab and typing “path”.
- Open the “qstart.m” and edit the run command so that it points to the file “qq\_startscript” (see highlighted line below).
- An example of “qstart.m” is shown below. The part to edit is shown in blue.

---

```

% Startupscript for Chrombox Q
% Starts startscript by the run command.
% Startscript must be located in the Q root,
% qstart must be in the matlab searchpath
% run c:\qq\qq_startscript
run /Users/yourname/Documents/CHROMBOX/qq/qq_startscript.m

```

---



## Linux computers:

- Download the installation and unzip the archive “[qq.zip](#)”
- Move folder “[QQ](#)” to the preferred destination, for example [\[/home/yourname/CHROMBOX/qq\]](#), This will be the Q-root folder
- The shell script [linstart\\_q.sh](#) stored in the Q-root folder can be used to start the program, if the file is executable and Matlab can be started with the terminal command “matlab”.
- On Ubuntu you can use the following procedure to make [linstart\\_q](#) executable:
  - Right-click on the file and select “Properties”. Select “Permissions” and “Allow executing file as program”.
- It should now be possible to start Chrombox Q by double-click on [linstart\\_q.sh](#) and selecting the option “run in terminal”. If you don’t get the “run in terminal” option while double-clicking the file you will have to edit the preferences in the file manager. Choose “Edit” in the menu for Files, thereafter “Preferences” and select the “Behaviour” tab. Select “Ask each time” as the option for executable text files.
- There is also a file [linstart\\_q\\_term.sh](#) in the Q-root folder. The difference between [linstart\\_q](#) and [linstart\\_q\\_term](#) is that [linstart\\_q](#) runs the application disconnected from the terminal while [linstart\\_q\\_term](#) runs in the terminal. Chrombox Q will continue to run if you close the terminal if it was initiated by [linstart\\_q](#), while it will close together with the terminal if it was initiated by [linstart\\_q\\_term](#).

As an alternative to the above procedure you can also start Chrombox Q by [qstart.m](#) as described above for Mac computers.

## Changing settings:

- The program should normally start without the need to change any settings. But you may want to adjust parameters such as window size. These are specified in the [qq\\_localsettings](#) file in the Q-root folder.
- Open the file “[qq\\_localsettings.sdv](#)” or “[qq\\_localsettings.csv](#)” in an editor such as Notepad and edit the paths for raw data, etc, if necessary.
- An example of “[qq\\_localsettings](#)” is shown below. Parts to check or edit are shown in blue.

---

[defaultfolders](#); [1](#); Specifies whether the default setup for folders is used (1 or 0, use 1 for simple setup)

[defaultmethod](#); [Default](#); Method to load on startup

[path\\_agilent](#); [C:\GC-MS-Data](#); Folder for MS chemstation rawdata

[path\\_cdf](#); [C:\GC-MS-Data](#); Folder for NetCDF (AIA format) rawdata

[path\\_export](#); [C:\CHROMBOX\qq\export](#); Folder for exported data

[path\\_library](#); [C:\CHROMBOX\qq\libraries](#); Folder for spectral libraries and data defining compound classes

[path\\_method](#); [C:\CHROMBOX\qq\methods](#); Folder for methods

[path\\_nist](#); [C:\NIST05\MSSEARCH](#); Folder NIST library

[path\\_qqraw](#); [C:\CHROMBOX\qq\rawdata](#); Folder for Chrombox Q raw data (Matlab format)



path\_reports; [C:\CHROMBOX\QQ\reports](#); Folder for Chrombox Q reports

path\_results; [C:\CHROMBOX\QQ\results](#); Folder for Chrombox Q results data

tracker; 0; For development purposes, 0 or 1

user; [Anonymous](#);

version; [Q-12-01](#); Code version to use

windowpos; [[0.025](#) [0.07](#) [0.95](#) [0.9](#)]; Window position and size [leftposition lowerposition width height] in fractions of screen size Notepad or win default

---

- If paths are left empty, e.g. “path\_agilent; ; Folder for MS chemstation rawdata“ the program assumes that the files are found in the corresponding folder under the Q-root folder, e.g. “C:\CHROMBOX\QQ\chemstation” for Agilent Chemstation data. It is therefore not necessary to specify paths unless they are different from the default setup.
- “windowpos” is position of the window in fractions of the screen size. The two first numbers in the vector is the position of the lower left corner. As specified above the lower left corner is 10% from the bottom of the screen and 10% from the left. The height and width is 75% of the screen size. Ensure that the sums of numbers 1 and 3 and numbers 2 and 4 are less than 1.
- If “defaultfolders” is set to 1 the program will use the standard setup for subfolders and it is not necessary to edit the paths even if they are not correct. If the parameter is set to 1 you will have to specify the location of each path for data and methods. Data can be read from other folders than the ones are specified. Folders can also be changed by using the [Settings] option within the program.
- “version” refers to the current version of the code. The parameter can also be updated from within the program.
- If you have created a method that you want to import on startup you specify this as defaultmethod.

### Using Chrombox Q with NIST MS-Search 2.0.

On Mac (OS X) and Linux, first install [Wine](#) to be able to run NIST MS-Search and verify that you can run windows exe-files. Thereafter proceed as described for Windows below. On Mac, the installer for [WineBottler](#) is a convenient way to install Wine. On Mac you currently need the beta version (Q-16-05b) of Chrombox to use the NIST MS-Search.

- Install the NIST software or copy an existing install (the NIST $xx$  folder with all its content) to a separate location, where  $xx$  refers to the library version, e.g. NIST05.
- Update the path to the NIST library in Chrombox Q. This can be done in the qq\_localsettings.sdv file or you can click [Settings] down in the right corner and choose [Paths / Version]. After editing the path to the NIST library you press [Save local settings]. The edit field for the path will be yellow if the path is not valid.



- If the file “autoimp.msđ” exists in the NISTxx\MSSEARCH folder, open it with a text editor (e.g. Notepad) and ensure that it points to an existing directory where you have write access.
- If the file don't exist, create a mass spectrum, for instance by double-click in a chromatogram in the main window of Chrombox Q. Thereafter right-click in the mass spectrum and select “Search NIST” from the menu. This should give you the option to create a new “autoimp.msđ”. Select [Yes] to create the file. You will typically get a warning message saying “NIST MS database directory invalid.”. Ignore this and press [OK]. Thereafter you will get the option to select the NIST MS database directory. Select the folder “MSSEARCH” under NISTxx and press [OK]. The program should open and display the search result.



## Chrombox Q – Tutorial 1

The main purpose of this tutorial is to get used to the most basic functions in the program. Fatty acid methyl esters (FAME) in a reference mixture will be identified using an existing libraries and an already calibrated method.

### 1.1. Startup and select method

- Start by opening the main window, by writing “qstart” in the Matlab command window or by starting the Chrombox Q.exe file in the Q root folder (i.e. C:\CHROMBOX\QQ). The main window should look as in Figure 1.1.
- Start by selecting the method “Tutorial-1”.
- Select [Load Data] which will take you to the main window for importing and converting raw data.

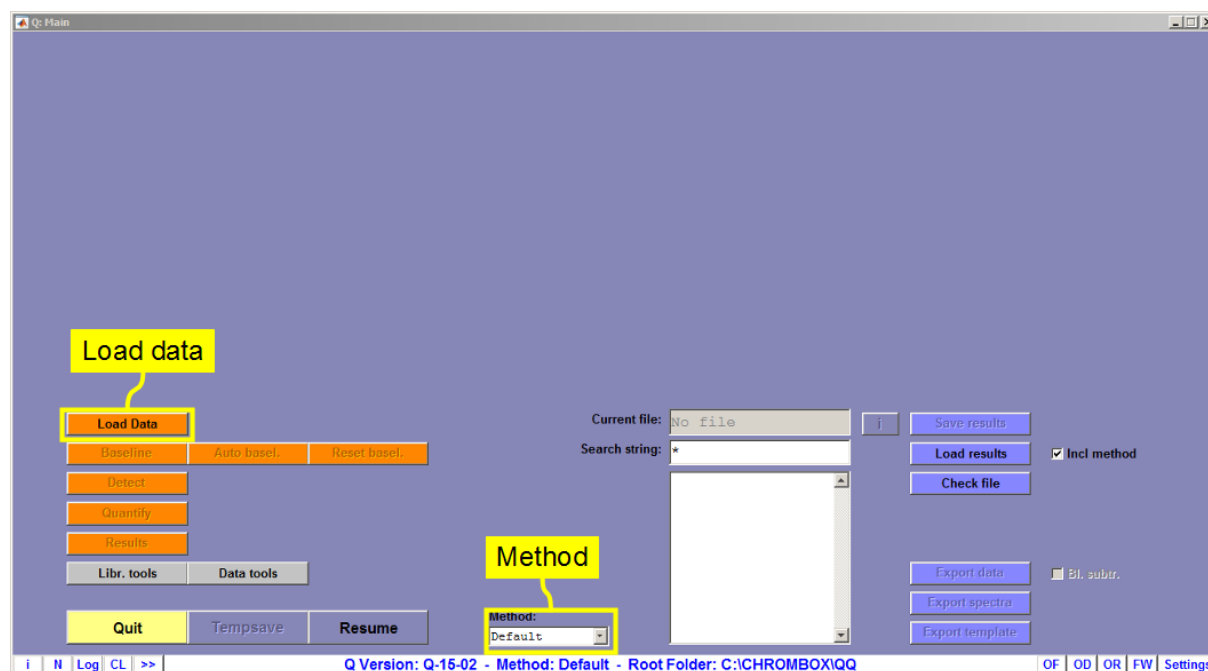


Figure 1.1. Main window at startup

### 1.2. Loading a file:

- The [Load] button takes you to the main window for loading raw data files
- Ensure that the current folder is the “TUTORIAL\_1-2” folder. If a different folder is selected you can navigate by pressing the [Dir Up] button or by right-click in a directory in the directory and files list. You can also scan for subfolders of the search path that contain the right data type by checking the “Scan” box next to the search path.
- Select the file “Tutorial-1” in the table in the middle
- Press [Load Sel.] and the file opens.



- Press thereafter [Accept] that will take you back to the main window.

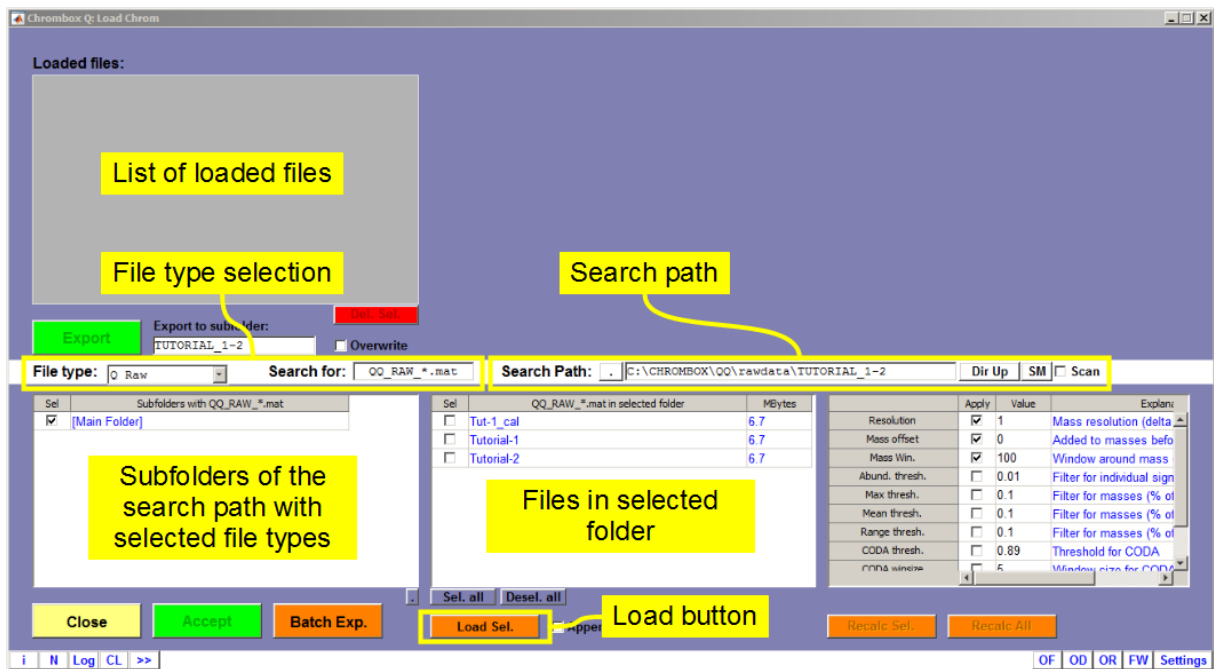


Figure 1.2. Load at startup

### 1.3. Baseline subtraction

- Before proceeding the baseline must be subtracted by pressing the [Auto Basel.] in the main window.
- After baseline subtraction the chromatogram should look like in Figure 1.3.

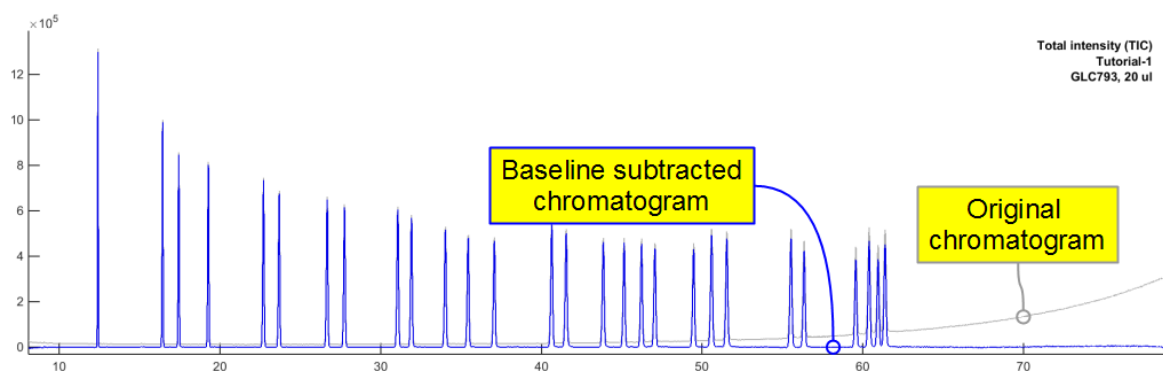


Figure 1.3. Chromatogram after baseline subtraction





## 1.4. Peak region detection

- The next thing you have to do is to detect the peaks in the chromatogram. Press [Detect] in the main window and the Peaks window shown in Figure 1.4 will open.
- Use the threshold slider to adjust the threshold line so that it is well above the baseline, but covers all peaks (see figure 1.4). You can also adjust the threshold by clicking on the red line.
- Press thereafter [Detect].
- Inspect all peaks. You can zoom in on a peak by clicking on it and thereafter pressing [(O)] followed by [+ ] on the zoom line under the chromatogram. You can navigate through the chromatogram by the slider on the zoom line or by the [<] and [>] buttons down in the right corner.
- If you are not satisfied with a peak region you can edit it by right-click on the rectangle marking the peak area or by clicking on the red vertical lines that mark the selected peak region.
- When all peaks are ok you zoom out by [-], press [Lock all] and thereafter [Accept].

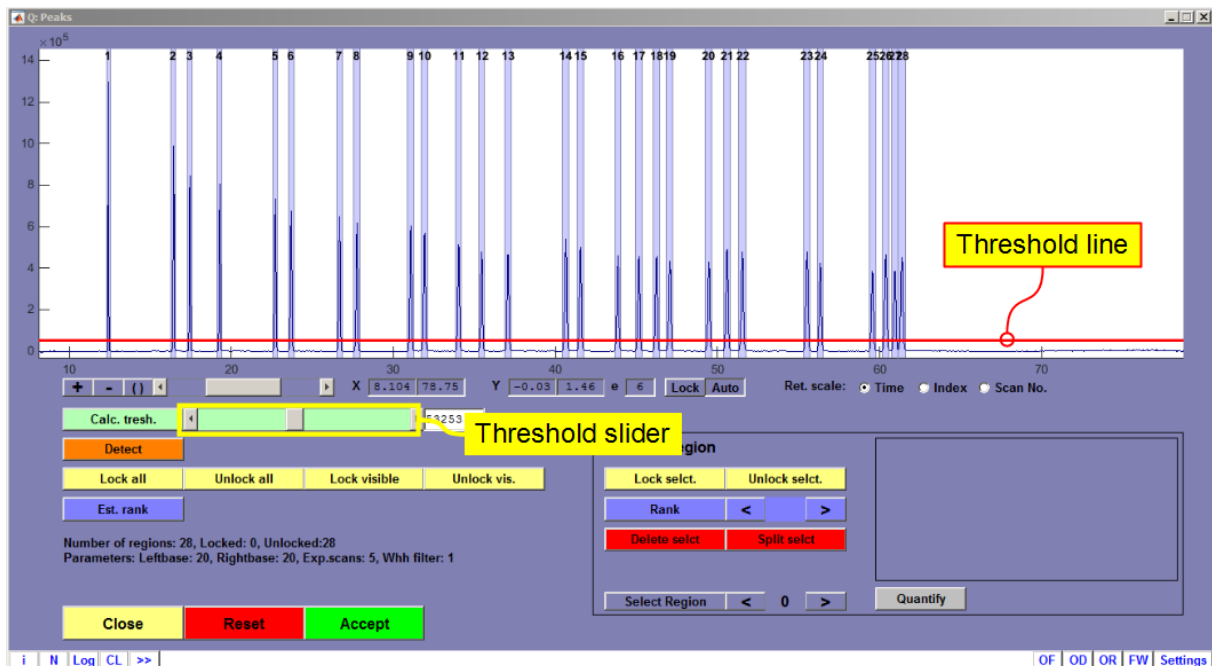


Figure 1.4. The peak detection window



## 1.5 Quantification

- After detection the peaks must be quantified. In this case the sample is a reference mixture where all peaks are resolved. The peaks can therefore be quantified by pressing the [All Single] button.
- Press [Close] when the last peak has been quantified.
- Press thereafter [Results] in the main window.

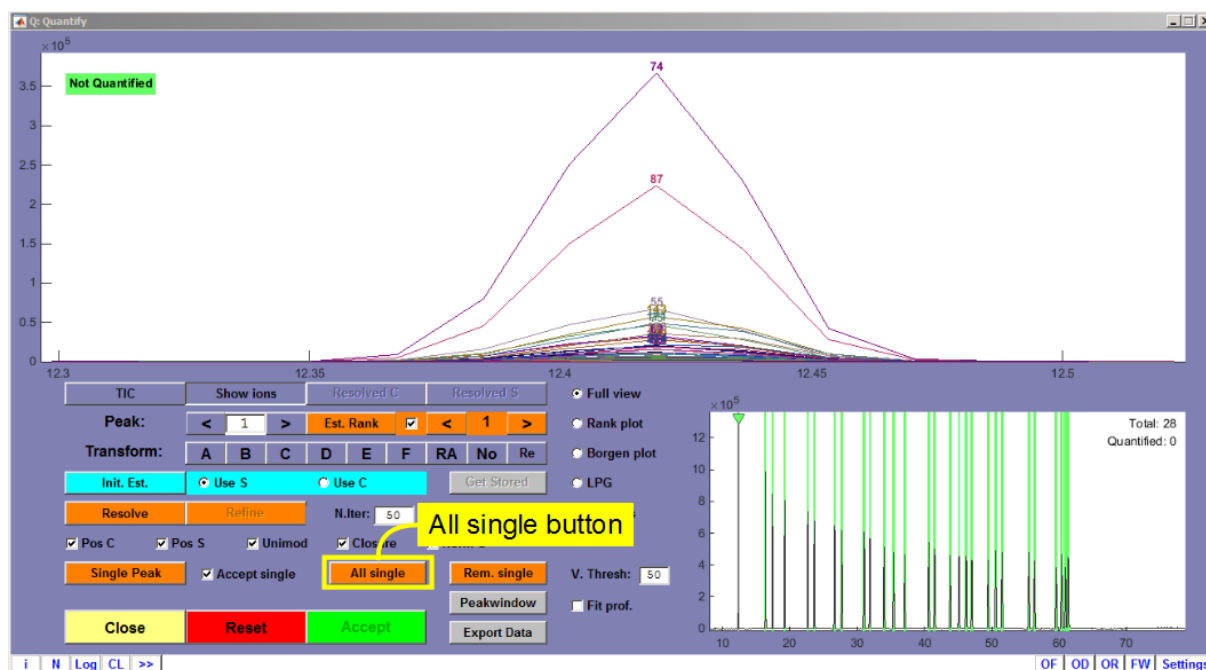


Figure 1.5. The Quantify window

## 1.6. Identification and reporting

- The results window is shown in Figure 1.6.
- The first thing you have to do is to calculate retention indices for the peaks. The retention indices for fatty acid methyl esters are equivalent chain lengths (ECL).
- The calibrations are stored in the method. Select the calibration “Tut-1\_cal” among the stored calibrations. Press thereafter the [Use Sel.] to activate the selected calibration.
- Press [Calc. RI] to calculate retention indices for the compounds in the mixture. By selecting “Ret. Index” as label you can view the retention indices for each peak. The ECL scale uses the saturated FAMES as calibration compounds. The saturated FAMES 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 23:0 and 24:0 should have ECL values close to integer values corresponding to the number of double bonds in the fatty acid chain. You can also choose to use retention index as x-axis instead of retention time by selecting the scale on the zoom line.
- When retention indices are calculated it is time to identify the compounds. First press [Settings] on the library line. Verify that “Z\_BP-20” is selected as search library. You can also add “HI-2009” if it is available. Check that “Gaussian function” is selected under



“Match retention” and that the interval is set to 0.9. Press thereafter [Close] and the libraries will be loaded to the memory.

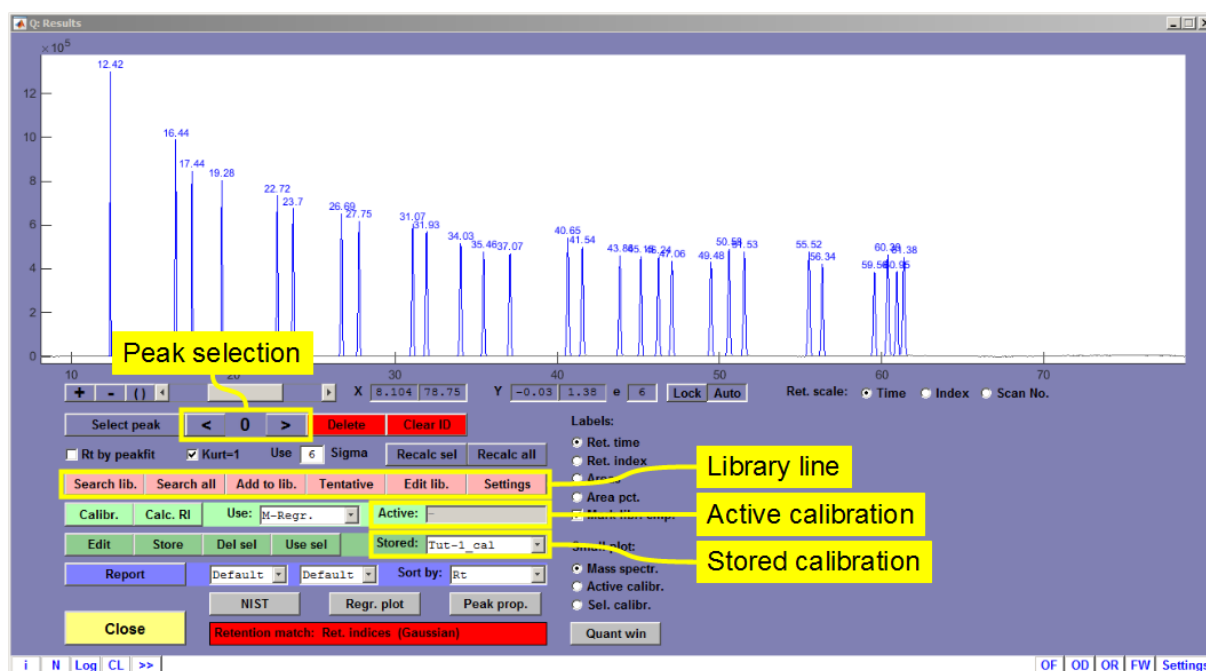


Figure 1.6. The Results window

- Select the first peak, either by clicking on it or by using the arrow-buttons [ $\leftarrow$ ] and [ $\rightarrow$ ]. This will show the mass spectrum of the compound.
- Press [Search Lib] to search the library. This will take you to the Identify window shown in Figure 1.7. The figure shows the results for the third peak (14:1 n-5).
- The compound list shows the 40 best matches from the library. The correlation plot shows similarity between the spectrum from the chromatogram and the library spectra. Ideally, all masses should be near the diagonal blue line.
- The match plot shows calculated scores for the compounds in the compound list in decreasing order. Compounds with the same identity as the spectrum selected in the list (the one with highest score by default) are shown in red. Other compounds are shown in blue. For a reliable identification the situation should be similar to the one shown in the figure, where all the best matches have the same identity, there is a clear difference to the next compound and the best matches are above the threshold value marked by the green field.
- The info box show additional info, such as the difference in retention indices and the source of the matching compound. In the list of search parameters you can set how the library search should be performed, for instance if and how retention indices should be used. By selecting “Exclude” instead of “Gaussian funct.” you can see the effect of omitting the retention indices and match the compounds using only spectra.
- Press [Accept] to accept the suggested or selected ID, [Close] to return without identifying the peak and [Reset] to delete the ID of an already identified peak.



- Go through all peaks in the chromatogram and identify them. Identified peaks will be drawn in green or red (if they are calibration compounds). Unidentified peaks are drawn in blue.
- If you are confident about the content of your sample you can also use the [Search all] option that will identify all peaks with match above the threshold, without opening the Identify window.

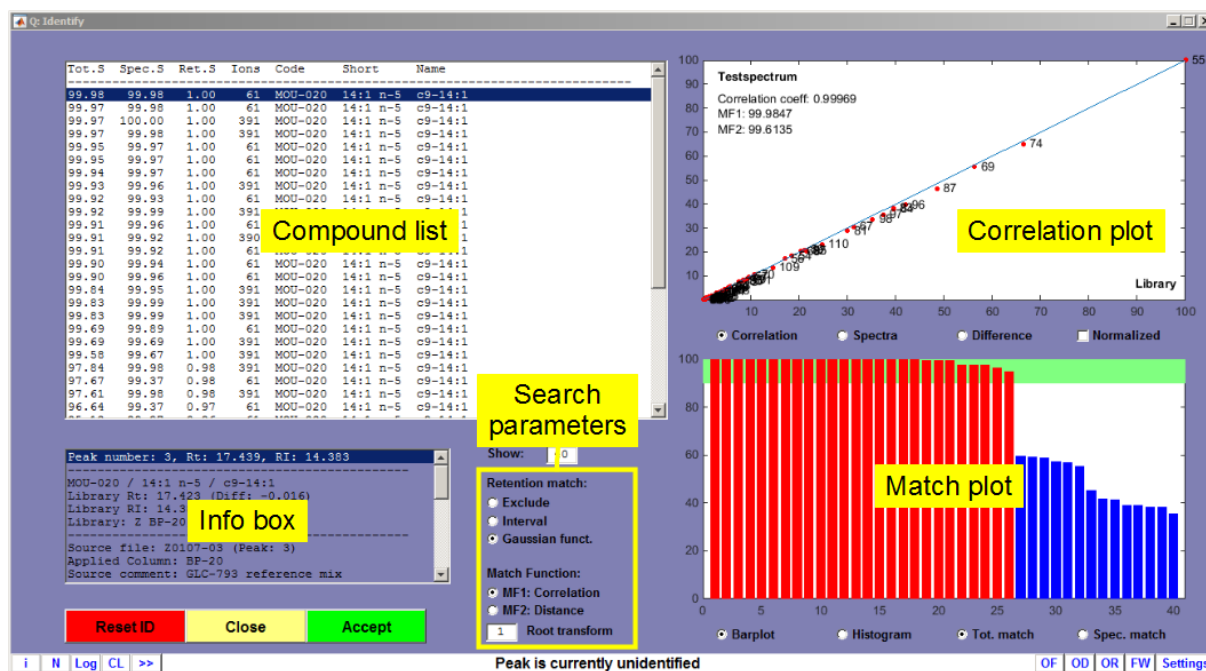


Figure 1.7. The Identify window

- When finished with the identification you can report the result by pressing the [Report] button. Various report formats can be selected in the field next to the button. A report in will open if the “Open” option is checked or if “Auto open” is set as default in the method.
- Press [Close] to leave the Results window.
- You can save your results by [Save results] in the main window and recall the results by typing “\*” or any relevant search string, such as “Tut\*” in the search string field, and thereafter import the selected file by the [Load] button.



## Chrombox Q – Tutorial 2

The purpose of this tutorial is to get used to peak detection and quantification in real samples, which usually have a more challenging peak pattern than reference mixtures. The sample is fatty acid methyl esters (FAME) from a fish oil. You will use the same method as in Tutorial 1.

### 2.1. Startup and select method

- Start the program, select the method “Tutorial-1”, load the file “Tutorial-2” and perform baseline subtraction in the same way as explained in Tutorial-1.
- After baseline subtraction the chromatogram should look like in Figure 2.1

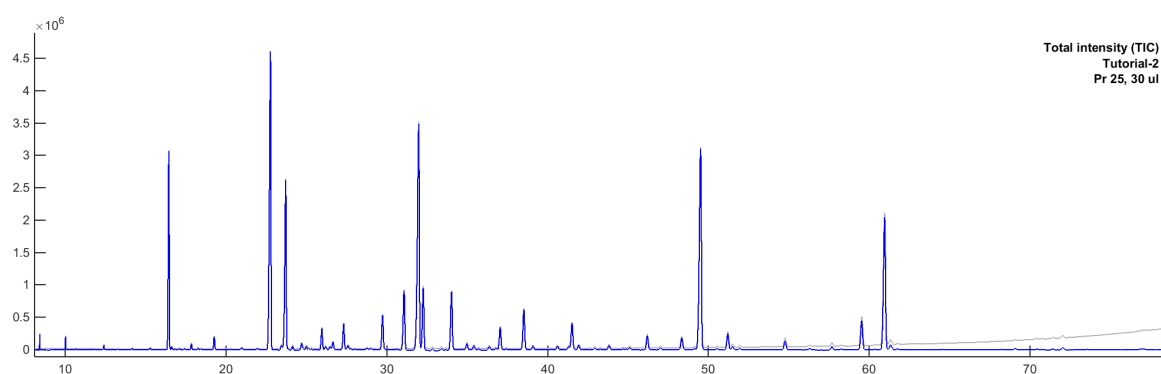


Figure 2.1. Chromatogram after baseline subtraction

### 2.2. Peak region detection:

- Press [Detect] in the main window, which will take you to the window for peak region detection.
- The challenge with this sample compared to the reference mixture is that there is large variation in the peak size. Some peaks are also partially overlapping. In the peak detection window the best way to detect the peaks is usually to start with a high threshold value and then gradually reduce the threshold.
- Start by adjusting the threshold slider so that the value is around 100 000 and press [Detect]. The result should look approximately like in Figure 2.2.

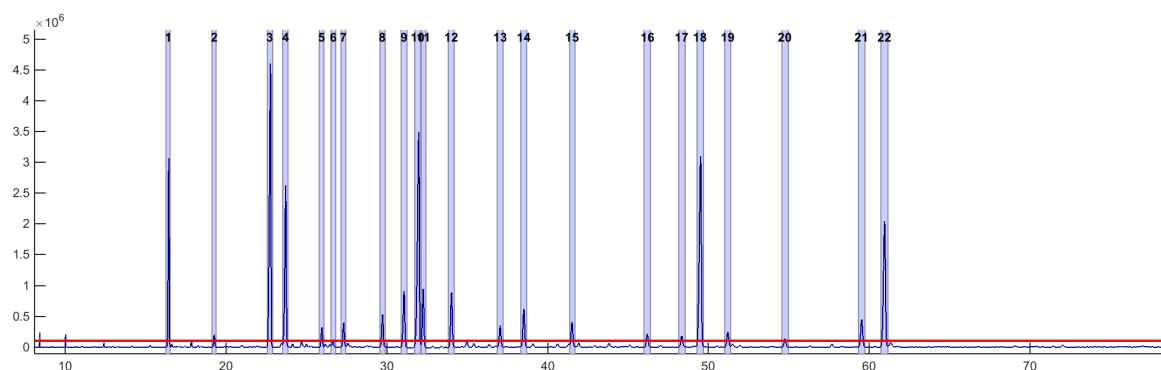


Figure 2.2. Peak region detection with a threshold of 100 000.



- Select the first peak, press the [()] button on the zoom line and zoom in on the peak. Verify that the marked area look reasonable and check all peaks by stepping through the chromatogram with the [>] button.
- When you have reached the last peak, press [Lock All]. This ensures that the already detected peak regions will not be affected by later detection of smaller peaks.
- Reduce the threshold to around 25 000.
- Zoom in on the chromatogram so that approximately 2-3 min is shown on the x-scale. Move to the start of the chromatogram using the slider on the zoom line. Adjust the Y-scale so that the threshold is clearly shown (-0.1 to 1 e<sup>5</sup>) are appropriate values. When you edit the Y-scale the scale will be locked at this level. You can toggle between locked mode and auto-scaling by the [Lock] and [Auto] buttons on the zoom line.
- Press [Detect Peaks]. You can adjust the selected regions by right-click in field that mark the regions or by first left-clicking on the field and thereafter clicking on the red vertical lines that mark the beginning and end of a peak region. The three first peaks should look approximately as shown in Figure 2.3.
- When you are satisfied with the peaks, press [Lock Visible] to protect the peaks. Be sure all the peaks you want to protect are visible in the window when you apply [Lock Visible]. An alternative to [Lock visible] is to lock the peaks one by one by [Lock Selct.].

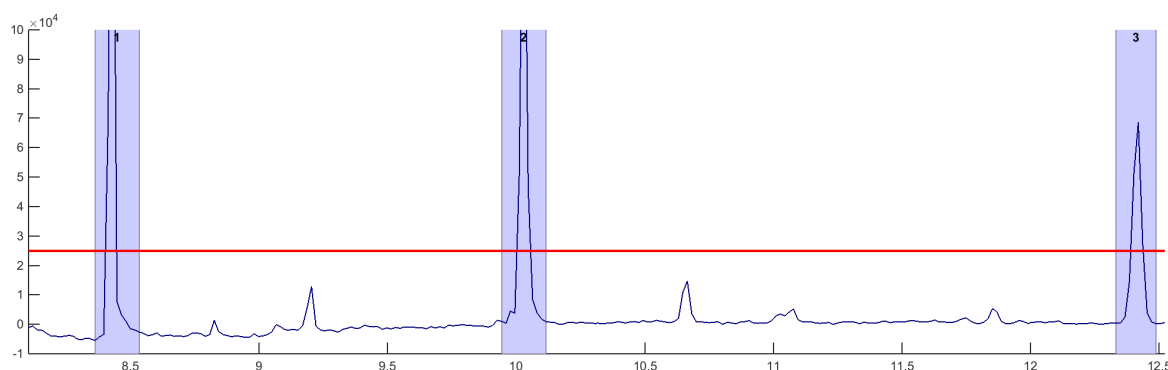


Figure 2.3. The three first peaks in unlocked mode.

- Scroll through the chromatogram and detect peaks that are above the threshold, adjust the marked area if necessary, and lock the peaks when the areas are ok. Some of the peaks will need adjustments of the areas.
- There are several alternative methods for adding and adjusting peak regions. If you left-click in the chromatogram above or below a peak while the shift button is pressed on the keyboard a peak region is added around the selected peak. Left-click in front or behind a single or a group of peaks while the ctrl button is pressed on the keyboard adds a region with user defined width. Right-click on the area that marks a peak region provides a menu with several more options.
- Right-click on the area that marks a peak region also provides options for viewing the average spectrum or the ion traces of the selected peak region.



- When the end of the chromatogram is reached there should be approximately 50 peaks. There are many minor peaks that are not detected at this level, but the threshold level is sufficient for this exercise.
- Zoom out to full view and verify that all peaks are locked (green areas). Press thereafter [Accept] to return to the main window.

### 2.3. Quantification

- Press the [Quantify] button to go to the Quantification window.
- Contrary to the reference mixture in Tutorial 1, where all chromatographic peaks were resolved, you may now have overlapping or partially resolved peaks. You must therefore evaluate in each case whether the peak is pure or not. In cases with overlapping peaks you have four possibilities
  - If there are parts of the cluster where pure spectra can be found you can resolve the cluster by using the estimates of the pure spectra (S)
  - If there are ions that are unique for the compounds you can resolve the peak cluster by using the selective ions (C)
  - If none of the two criteria above are fulfilled, but there is a clear valley between overlapping peaks you can go back to the peak cluster by pressing the [Peakwindow] button and split the peak
  - If none of the three criteria above are fulfilled the cluster cannot be resolved and you must quantify it as a single peak or use a manual split in the peak window.
- The first peaks are typically pure peaks. Quantify these by pressing the [Single Peak] button. If the 'Accept single' option next to the button is active the program moves to the next peak. If this is off you have the possibility to view the spectrum by pressing the [Resolved S] button after quantification, but you must press the [Accept] button to accept the solution.
- At approximately 21 minutes there is a peak with a deviating peak shape with a shoulder to the left, but the two overlapping peaks have nearly identical spectra and there is no valley between them (Figure 2.4). The only option is therefore to quantify them as one peak or to jump to the peak window and split the peak manually.

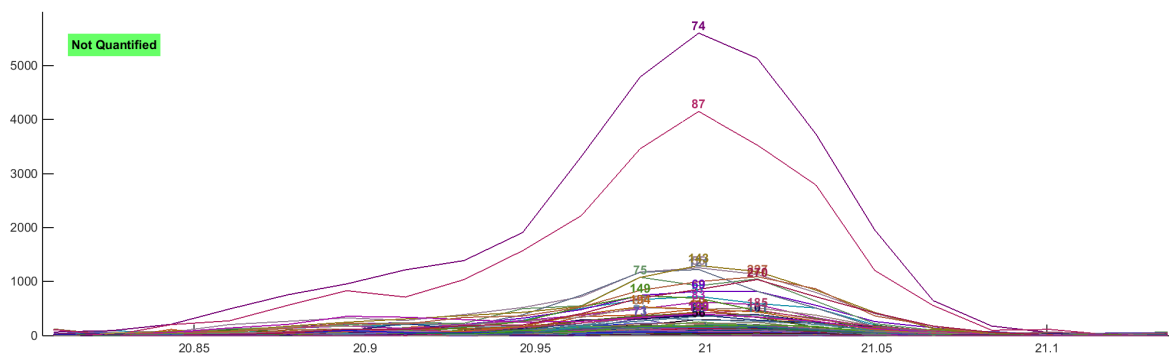


Figure 2.4. Overlapping peaks with highly similar spectra



- The next peak cluster is found around 24.7 min. In this case there is a clear shoulder to the right, but there are also impurities in the main peak. If you press the button [Init Est.] you will get a plot similar to Figure 2.5 where the program tells where the purest spectra are likely to be found (the vertical lines) and which ions that may be selective (thick lines). The minor impurity under the main peak is not even partially resolved. The spectra can therefore not be used for resolution, but you may use the ions by selecting 'Use C' followed by the [Resolve] button. The suggested chromatographic solution is shown. If you inspect the spectra by pressing [Resolved S] you will see that there are negative ions, which means that the solution is not good. Press the [Refine] button to refine the solution. You should then get spectra similar to the ones in Figure 2.7. By right-clicking in the spectra you can do a preliminary search in the libraries to check that the spectra match any known compounds.
- Press [Resolved C] to check that the resolved chromatographic profiles make sense. The blue profile is flat and wide because the peak actually consists of two isomers (*cis* and *trans* 7-methyl-6-Hexadecenoic acid) that cannot be distinguished by mass spectrometry.
- Press [Accept] to move to the next peaks.

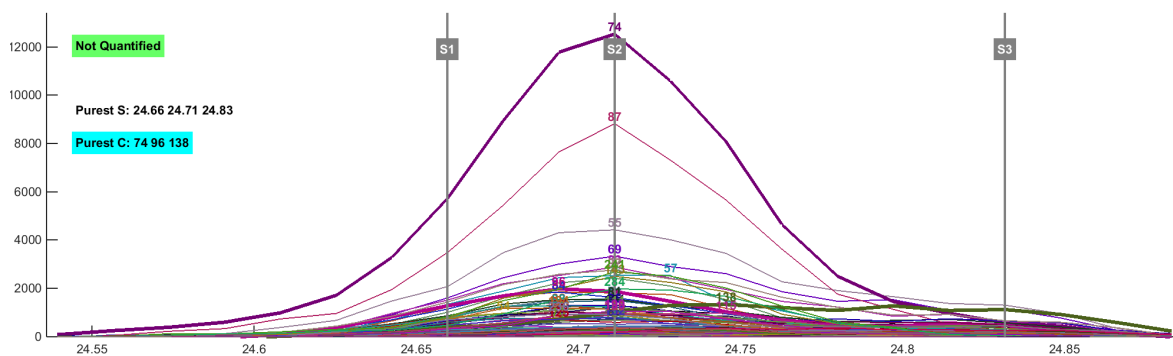


Figure 2.5. Initial estimates of peak cluster at around 24.7 min.

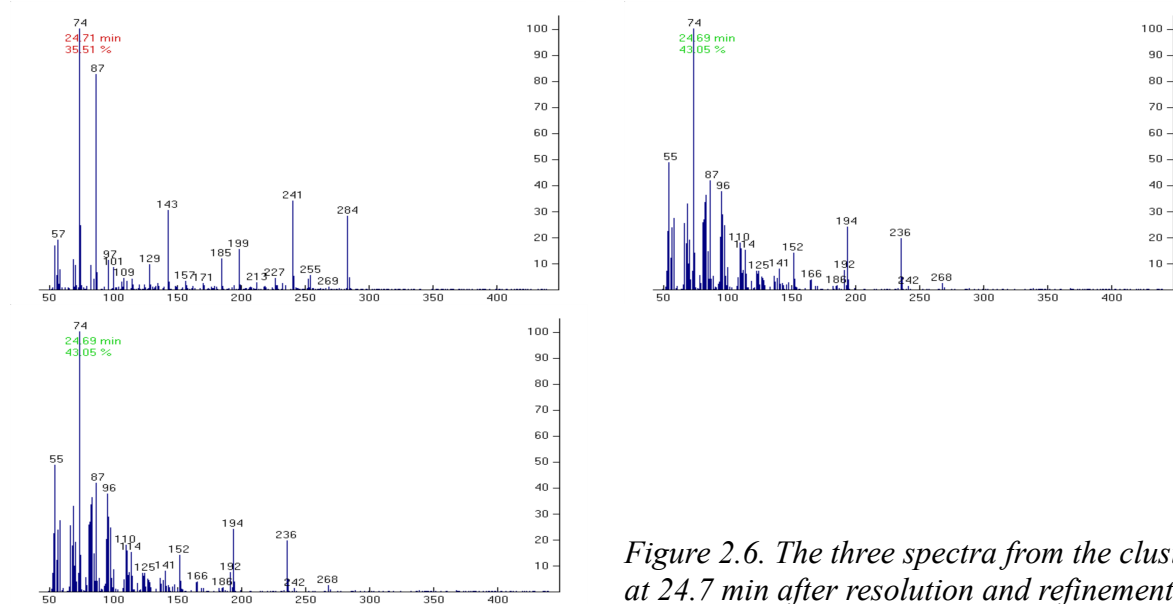


Figure 2.6. The three spectra from the cluster at 24.7 min after resolution and refinement.





- At around 27.7 there is a new peak cluster that clearly consists of two peaks that are partially resolved (Figure 2.7). When peaks are only partially overlapping like this the best strategy is usually to use the estimates of the spectra for resolution.
- Press [Init Est.] followed by 'Use S' and [Resolve] to quantify the two peaks.
- Press [Accept] to move on to the next peaks.

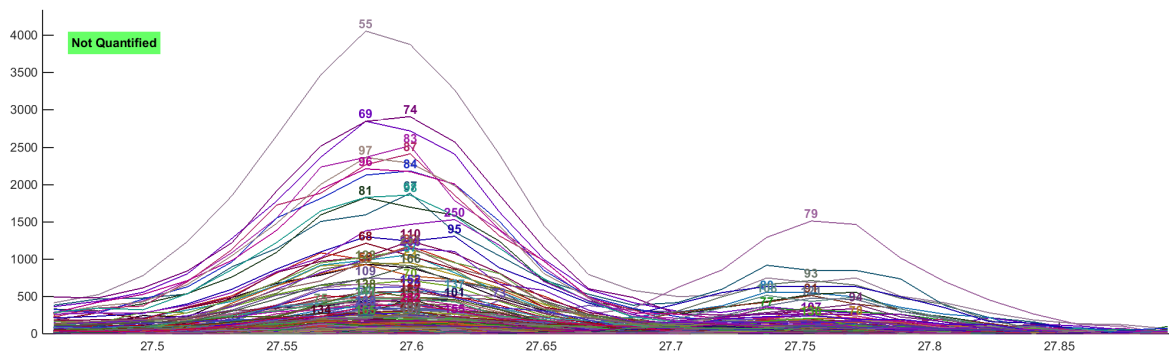


Figure 2.7. The peak cluster at around 27.6 min.

- At around 36.3 there is a new cluster of two severely overlapping peaks (Figure 2.8). In this case there are selective ions but the peaks are too overlapping to give good estimates of the spectra. Press [Init Est.], 'Use C', followed by [Resolve]. Press [Resolved S] to inspect the spectra. If you now press [Refine] you will see that the spectra are confounded again (the masses 185 and 187 appear in the spectrum to the left). In some cases you may get better results by omitting the Refine procedure. It is therefore often necessary to evaluate the results before and after [Refine].
- In this case you will get the most accurate spectra by doing a refinement without the 'Closure' option. Repeat the procedure [Init Est.] and [Resolve]. Deselect the Closure option and press [Refine]. Press [Resolved S] to inspect the spectra again. Deselecting the closure option may give you more accurate spectra, but in some cases you may get worse estimates of the chromatographic areas.
- Press [Accept] and move on to the next peaks.

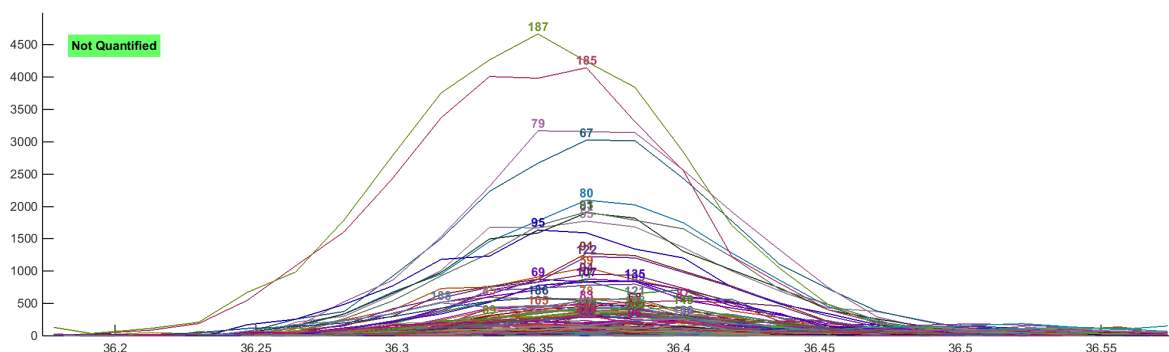


Figure 2.8. The peak cluster at around 36.3 min.



- At approximately 48.4 min there is a new cluster with one large and one small peak. There is a quite good resolution between the peaks and this cluster is best resolved using the spectra. Press [Init Est.], 'Use S', [Resolve] and [Refine] (with Closure activated). The minor peak is from a furan fatty acid. You can verify this by doing a preliminary search in the library (right-click on the spectrum).
- The remaining peaks are rather pure and can be quantified as single peaks.
- When you reach the last peak, press [Close] to get back to the main window.

#### 2.4. Identification and reporting

- Press [Results] to get to the Results window.
- Select the calibration 'Tut-1\_cal' and press [Use Sel.]. Press thereafter [Calc. RI] to calculate retention indices. Select 'Ret. Index' as labels and check that the index for the highest peak is around 16.0. This is the 16:0 FAME, which has ECL value of 16 by definition.
- Press [Settings] and check that you have activated the same libraries as in Tutorial 1, that retention scale is retention index, and that 'Match Retention' is set to Retention index. Press [Close] and the libraries will be loaded to memory.
- You can now try to identify the peaks by using the [Search All] button. It may still be necessary to check the identities of the peaks. If any labels are marked orange it means that a compound was identified twice. Large peaks may be skewed and get inaccurate retention indices.
- In the beginning of the chromatogram there are two minor peaks that were not identified. These are the saturated FAMES 8:0 and 10:0. These are outside the range of the retention index calibration that starts at 12:0. You can identify these by searching only the spectra. Select one of the peaks and press [Search Lib]. In the Identify window, select 'Exclude' under 'Retention match' and accept the result if it is correct. Repeat for the other peak.
- You can now press [Report] to report the results, which should look approximately as in the table below (with additional columns for peak width and asymmetry).

Rt	Retind	Code	Short	Name	Area	A pct
8.4306	9.3433	SAN-001	8:0	8:0	299990	0.159
10.0295	10.4889	SAN-002	10:0	10:0	318573	0.1688
12.4192	12.0001	SAN-003	12:0	12:0	169627	0.0899
15.2902	13.4971	SAB-242	i-14:0	12-Methyltridecanoic acid	102872	0.0545
16.4592	14.0071	SAN-005	14:0	14:0	12628513	6.693
16.6312	14.0769	SAB-324	4,8,12-Me 13:0	4,8,12-trimethyl tridecanoic acid	166059	0.088
17.8518	14.5282	SAB-078	i-15:0	13-Methyltetradecanoic acid	364883	0.1934
19.2787	15.0000	SAN-006	15:0	15:0	888811	0.4711
20.9979	15.5221	SAB-072	i-16:0	14-Methylpentadecanoic acid	192774	0.1022
22.7686	16.0141	SAN-007	16:0	16:0	29922011	15.8585
23.4563	16.1982	MOU-275	16:1 n-9	c7-16:1	375351	0.1989
23.7142	16.2657	MOU-021	16:1 n-7	c9-16:1	15749982	8.3474
24.144	16.3766	MOU-255	16:1 n-5	c11-16:1	188273	0.0998
24.6941	16.5159	MOU-297	16:1 n-x	x-16:1	325894	0.1727
24.7113	16.5202	SAB-074	i-17:0	15-Methylhexadecanoic acid	268821	0.1425
24.7457	16.5288	MOB-289	16:1 n-10, 7Me (a)	7-methyl-6-Hexadecenoic acid (a)	162235	0.086



25.0036	16.5931	DIU-091	16:2 n-7	c6,c9-16:2	256582	0.136
25.9663	16.8294	DIU-201	16:2 n-4	c9,c12-16:2	1812954	0.9609
26.1898	16.8837	MOB-286	16:1 n-10, 7Me (b)	7-methyl-6-Hexadecenoic acid (b)	294282	0.156
26.4649	16.9502	SAB-071	Phytanic acid	3,7,11,15-Tetramethylhexadecanoic acid	269995	0.1431
26.654	16.9959	SAN-008	17:0	17:0	720402	0.3818
27.3245	17.1558	POU-046	16:3 n-4	c6,c9,c12-16:3	2391560	1.2675
27.5995	17.2204	MOU-436	17:1 n-8	c9-17:1	445910	0.2363
27.7715	17.2606	POU-049	16:3 n-3	c7,c10,c13-16:3	97583	0.0517
29.7313	17.7064	POU-052	16:4 n-1	c6,c9,c12,c15-16:4	3425987	1.8158
31.0723	18.0038	SAN-009	18:0	18:0	6373813	3.3781
31.9835	18.2031	MOU-023	18:1 n-9	c9-18:1	27737799	14.7009
32.2585	18.2625	MOU-079	18:1 n-7	c11-18:1	6203432	3.2878
34.0293	18.6396	DIU-027	18:2 n-6	c9,c12-18:2	6192620	3.2821
34.9748	18.8382	DIU-158	18:2 n-4	c11,c14-18:2	636774	0.3375
35.4218	18.9317	POU-030	18:3 n-6	c6,c9,c12-18:3	363527	0.1927
36.3502	19.1253	NFA-288	Unknown	Unknown	132094	0.07
36.3846	19.1325	POU-050	18:3 n-4	c8,c11,14-18:3	229524	0.1216
37.0551	19.271	POU-032	18:3 n-3	c9,c12,c15-18:3	2402101	1.2731
38.5164	19.5703	POU-053	18:4 n-3	c6,c9,c12,c15-18:4	4490449	2.3799
39.1009	19.6893	POU-056	18:4 n-1	c8,c11,c14,c17-18:4	384817	0.204
40.6138	19.9965	SAN-011	20:0	20:0	375761	0.1992
41.3014	20.1359	MOU-097	20:1 n-11	c9-20:1	314863	0.1669
41.5249	20.1811	MOU-024	20:1 n-9	c11-20:1	3111418	1.649
41.9375	20.2643	MOU-087	20:1 n-7	c13-20:1	476747	0.2527
43.8114	20.6411	DIU-028	20:2 n-6	c11,c14-20:2	485828	0.2575
45.1008	20.8999	POU-033	20:3 n-6	c8,c11,c14-20:3	242551	0.1286
46.2011	21.1208	POU-035	20:4 n-6	c5,c8,c11,c14-20:4	1642554	0.8705
48.3329	21.5476	POU-054	20:4 n-3	c8,c11,c14,c17-20:4	1452873	0.77
48.4876	21.5786	FUR-189	DiMeF(11,3)	13,14-Dimethyl-12,15-epoxyoctadeca-12,14-dienoic acid	48839	0.0259
49.5191	21.7854	POU-036	20:5 n-3	c5,c8,c11,c14,c17-20:5	27143309	14.3858
51.2039	22.125	MOU-262	22:1 n-11	c11-22:1	1971875	1.0451
51.4962	22.1841	MOU-025	22:1 n-9	c13-22:1	436845	0.2315
54.7626	22.8468	POU-069	21:5 n-3	c6,c9,c12,c15,c18-21:5	1131645	0.5998
57.6681	23.4415	POU-066	22:5 n-6	c4,c7,c10,c13,c16-22:5	346062	0.1834
59.5248	23.825	POU-038	22:5 n-3	c7,c10,c13,c16,c19-22:5	3758989	1.9922
60.9517	24.1218	POU-039	22:6 n-3	c4,c7,c10,c13,c16,c19-22:6	17701834	9.3819
61.3299	24.2007	MOU-026	24:1 n-9	c15-24:1	782765	0.4149
72.0061	26.4847	OTH-178	Cholestadiene	Chelesta-x,x-diene	269605	0.1429



## Chrombox Q – Tutorial 3

The purpose of this tutorial is to learn advanced baseline subtraction using CODA, building a retention index calibration from scratch, and use the calibration to identify compounds in a mixture of PAH.

### 3.1. Startup, select method and load alkane sample

- Start the program, select the method “Tutorial 3”, load the file “ALKANES” and return to the main window by pressing [Accept].
- The chromatograms show a dilute sample of every n-alkane from C9 to C40. The last compounds are completely hidden in the column bleed.

### 3.2. Baseline removal

- Open the baseline window by pressing [Baseline] in the main window.
- The window should look like in Figure 3.1. Important functions are CODA and the baseline finder. CODA [Windig et al. Anal. Chem. 68 (1996) 3602)] is a filter for removal of ions that are basically found in the background. The baseline finder is a background subtraction function.

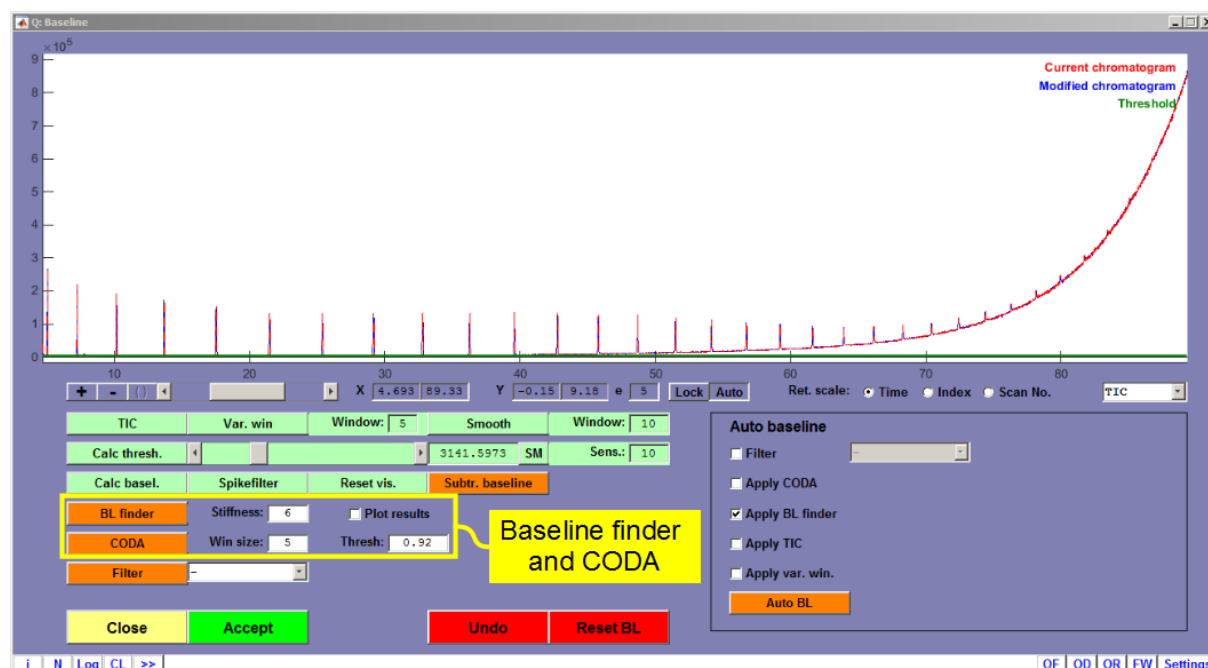


Figure 3.1. The baseline subtraction window

- Press [CODA] using a threshold level of 0.8. This will remove approximately 320 ions of the original 526 ions in the raw data, but it has limited influence on the column bleed.
- Gradually increase the threshold level in steps of 0.02 and press CODA after each step. You should see a gradual improvement in the background level as more ions are removed.



- At a threshold level at 0.92 you will see a dramatic reduction of the background when the ions from the column bleed are filtered away, and small peaks at the end of the chromatograms will be visible.
- The chromatogram should now as in Figure 3.2

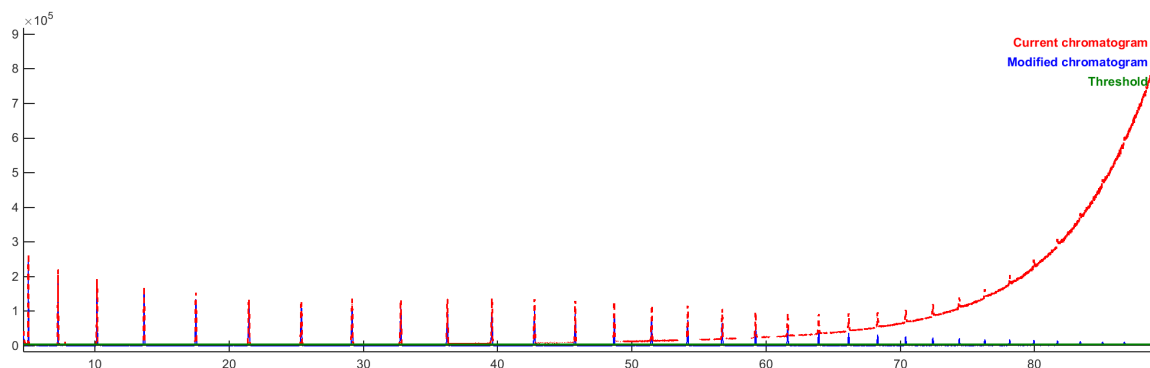


Figure 3.2. Chromatogram after filtering with CODA using window size 5 and a threshold of 0.94

- After filtering by CODA you thereafter press [BL Finder] to subtract the background of remaining ions.
- Press thereafter [Accept].

### 3.3. Peak region detection:

- Press [Detect] in the main window, which will take you to the “Peaks” window.
- Peaks are detected as in Tutorial 1 and 2. A threshold level of approximately 5000 is suitable.
- It may be necessary to adjust the borders of the last peaks that is poorly separated from the noise. Click on the last peak. Select [()] on the zoom line and zoom in on the peak using [+]. Right click on the green field and select “move borders” to adjust the start and end of the peak. Repeat for the second last peak and scroll thereafter through the chromatogram by the [<] button and adjust peaks if required.
- Zoom thereafter out to full view and check that you have 32 peak regions that appear with fairly regular intervals. The detected peaks should look like in Figure 3.3.
- Press thereafter [Accept].

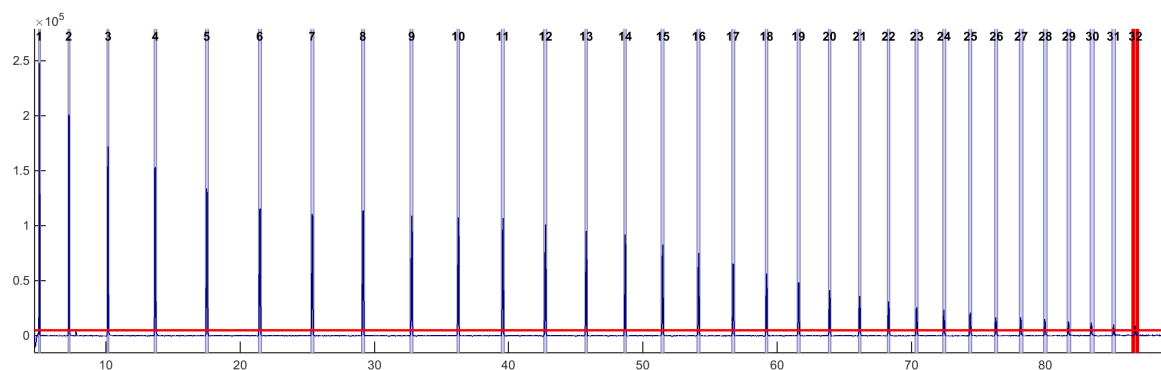


Figure 3.3. Detected alkanes after adjustment of peak widths



### 3.4. Quantification

- Since the sample is a reference mixture that contains no peak overlap you can quantify the peaks by pressing [Quantify] in the main window and [All single] in the quantify window. Press [Close] when the last peak has been reached.

### 3.5. Building the calibration.

- Press [Results] to open the results window.
- Press thereafter [Settings] and make sure the following are set:
  - Libraries to search in should be only “Alkanes”.
  - The “Store spectra in RAM” option should be selected. This usually speeds up library searches because the libraries do not have to be read from disk each time a search is performed.
  - Retention scale should be “Retention index”.
  - Since no retention indices are yet calculated, Match retention must be set to “Exclude”.
  - Interval should be set to 90. This is 100 times higher than the interval used in the previous tutorials because you will now use Kovats’ indices where the distance between homologous n-alkanes are 100, while the distance is 1 for the ECL scale used with FAME.
  - “Root” should be set to 2. This will take the square-root of all the spectra before they are compared, which will increase the influence of minor ions in the spectra. Negative signals in the spectra are also deleted when root is set higher than 1.
  - Press [Close] when settings are appropriate.
- Select the first peak. This should be the C9 alkane with a visible molecular ion of 128.
- Press thereafter [Search Lib]. The peak should be clearly identified as n-nonane. Press [Accept] to accept the result.
- Since the sample contains all n-alkanes from C9 to C40 the next peaks should be C10, C11, C12, etc. Identify these the same way as n-nonane.
- As the chain length increase the spectra will be more similar and the molecular ion may be weak or absent. For the last compounds you may have to look further down in the list to find the right compound. A way to avoid that is to follow the procedure below:
  - After for instance C20, when the spectra are becoming more similar, Press [Calibr] and [Calc. RI] in the Results window. This will give you a temporary calibration where the next peaks can be identified by extrapolation.
  - Select the next unidentified peak. Press [Search Lib] and select “Gaussian function” under “Retention match”
  - This should give you a clear result because retention indices are now applied in addition to the spectra.
  - When the identities start to become less clear (for instance with score values less than 95) you repeat the procedure with [Calibr] and [Calc. RI] and continue.



- When the last compound is identified press [Calibr] and [Calc. RI] again. You should then have a calibration that shows a fairly linear relationship between retention index and retention time (Fig. 3.4).
- Press [Store] to store the calibration in the method
- Close the window and press [Save Results].
- Since you may need the calibration later you must also save the updated method with the calibration. Do this by selecting [Settings] in the lower right corner of the main window. Press thereafter [Save Meth.] followed by [Close].

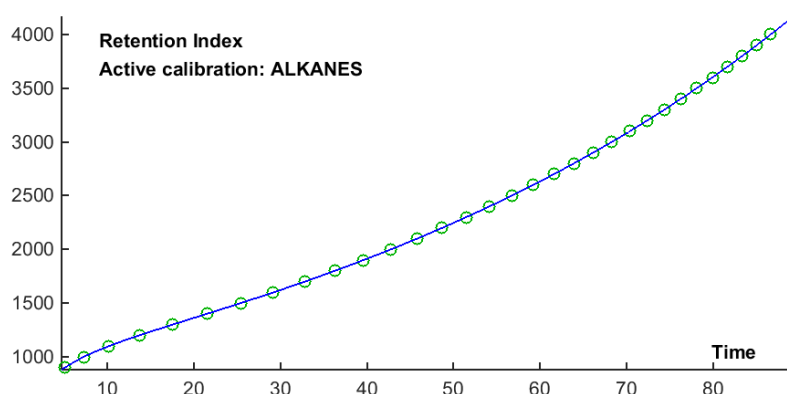


Figure 3.4. Final calibration

### 3.6. Loading the PAH-mixture

- Press the [Load] button and import the file “PAHMIX”

### 3.7. Background removal

- Press [Baseline] to go to the baseline window. Press [CODA] using the same parameters as last time and press thereafter [BL Finder].
- Press [Accept] to accept the solution and return to the main window.

### 3.8. Peak region detection

- Press [Detect] to go to the Peak detection window.
- Set the threshold level to approximately 40000 and detect the peak regions by pressing the [Detect] button.
- Select the first peak and zoom in on this using the [()] and the [+] buttons.
- Any peaks before 10 minutes are not PAH and should be deleted using the [Delete selct.] button.
- Scroll through the chromatogram using the [>] button and check that starts and ends of the peaks are properly set. Adjust if necessary.
- Press [Accept] when finished.



### 3.9. Quantification

- Press [Quantify] that will take you to the quantification window and quantify the peaks.
- There are several overlapping peaks. Proposed solutions are given below:
- Two-peak cluster from approx. 21.55-21.80 min: This is two peaks with very different spectra. It can easily be resolved by pressing the [Resolve] button followed by [Accept].
- Two-peak cluster from approx. 32.40-32.70 min: Same as above
- Two-peak cluster from approx. 37.40-37.75 min: This is of two peaks with almost identical spectra and deconvolution will not work since it requires differences in the spectra. The best way to solve it is to press the [Peakwindow] button that will take you back to the Peak detection window. Press [Split Select] to split the peak. Press thereafter [Accept], which will take you back to the quantification window. You can now quantify the cluster as two single peaks.
- Two-peak cluster from approx. 38.10-38.45 min: Same solution as above.
- Two-peak cluster from approx. 42.15-42.55 min: This is of two peaks with different spectra and the peak overlap is small. Both [Resolve] and splitting the peak in the peak window will work. Using [Resolve] is usually the simplest solution.
- Two-peak cluster from approx. 46.80-47.10 min: This is a problem with two severely overlapping peaks and no differences in the spectra. There is no ideal solution for these cases, but the best option is split it in the Peak window and quantify the two parts as single peaks. Areas may not be accurate but spectra and retention times will usually be ok.
- Two-peak cluster from approx. 53.05-53.40 min: This is of two peaks with similar spectra and the peak overlap is small. Splitting the cluster in the Peak window and quantifying the two peaks individually is the best option.
- Tree-peak cluster from approx. 60.50-61.05 min: This is a problem with two peaks with similar spectra and one peak with different spectra. There are two solutions for this. You can go to the peak window and split the last peak from the two other. This will give you a peak cluster consisting of two peaks with different spectra that can be resolved by pressing the [Resolve] button and one isolated peak that can be quantified as single. The other option is to press [Resolve] with all three peaks in the cluster, thereafter right-click on the profile with two peaks. Choose “Select split position”, place the axis-cross in the valley and press the left mouse button. This will give you three peaks (as shown in figure 3.5). Note that if you use [Refine] before the peak splitting you must turn off the “Unimod” (unimodality) option, since this option forces profiles to have only one maximum.
- Press [Close] when the last peak/cluster is quantified.





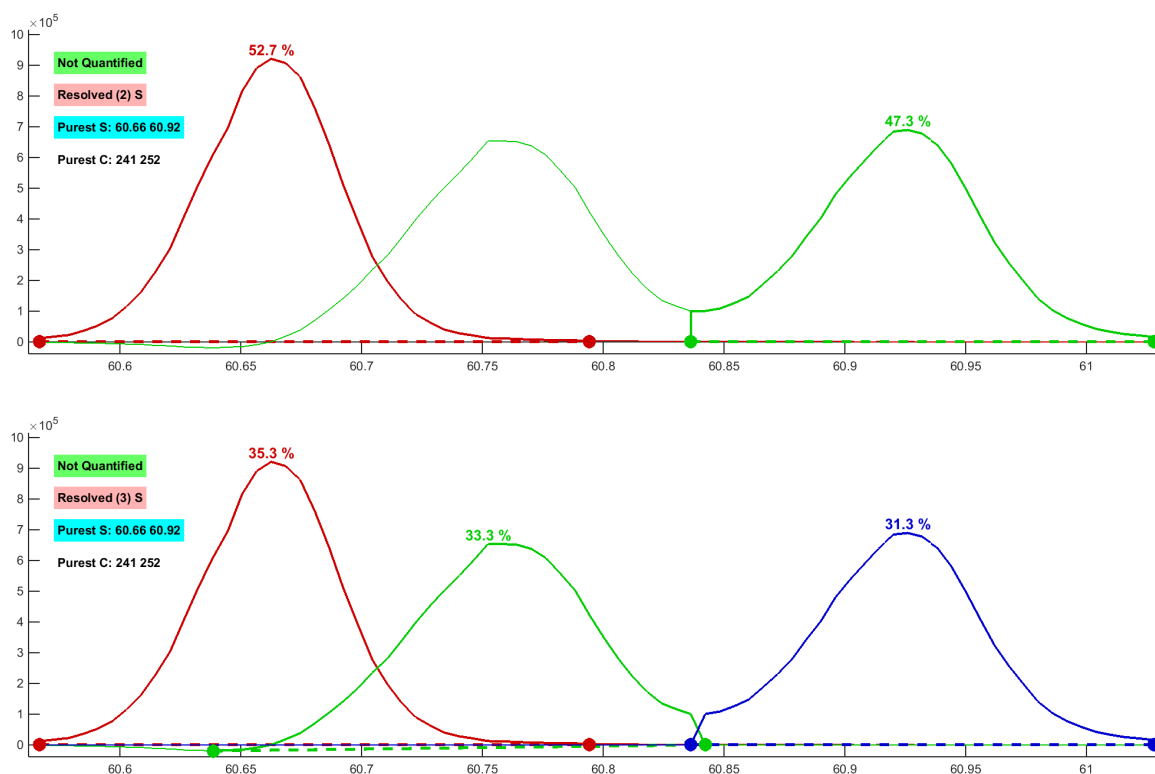


Figure 3.5. The three-peak after resolve and after splitting the double-peak.

### 3.10. Identification

- Press the [Results] button in the main window
- Select your stored calibration “ALKANES” and press [Use Sel]. Press thereafter [Calc RI] to calculate retention indices for each peak. Change the labels to retention indices.
- Press the [Settings] button and to the following: Select “POP-Rxi-1”, which is the right library for the applied column, as the only library in the “Search in” list. The library contains spectra and retention indices of various organic environmental pollutants.
- Check that “Match retention” is set to “Gaussian function”, that “Retention scale” is set to “Retention Index”, that “Spectr. Searchparam” is set to “MF1: Correlation” and that “Interval” is set to 90. Close the settings window.
- You can thereafter identify the peaks one by one or by the [Search All] button.
- Press the [Report] button with the options “TXT” and “Open”. Inspect your report to verify that all peaks are different PAHs. The list of expected compounds is shown in Table 1.



Table 1: list of expected compounds.

Rt	Retind	Code	Short	Name
12.0939	1156.8524	PAH-138	CAS: 91-20-3	Naphthalene
16.1506	1264.9268	PAH-144	CAS: 91-57-6	2-Methylnaphthalene
16.7126	1279.2353	PAH-139	CAS: 90-12-0	1-Methylnaphthalene
20.3976	1372.6646	PAH-140	CAS: 581-42-0	2,6-Dimethylnaphthalene
20.9303	1386.1244	PAH-141	CAS: 575-41-7	1,3-Dimethylnaphthalene
21.6218	1403.6813	PAH-142	CAS: 571-58-4	1,4-Dimethylnaphthalene
21.7366	1406.5986	PAH-143	CAS: 208-96-8	Acenaphthylene
23.1115	1441.5578	PAH-100	CAS: 83-32-9	Acenaphthene
25.0985	1492.5189	PAH-232	CAS: 2131-38-6	1,3,7-Trimethylnaphthalene
26.4633	1528.1075	PAH-233	CAS: 2245-38-7	2,3,5-Trimethylnaphthalene
26.8127	1537.2766	PAH-101	CAS: 86-73-7	Fluorene
27.8478	1564.6233	PAH-234	CAS: 879-12-9	1,2,3-Trimethylnaphthalene
31.0076	1650.1296	PAH-235	CAS: 13764-18-6	1,4,6,7-Tetramethylnaphthalene
32.5222	1692.2537	PAH-236	CAS: 2131-43-3	1,2,5,6-Tetramethylnaphthalene
32.6032	1694.5307	PAH-176	CAS: 132-65-0	Dibenzothiophene
33.5456	1721.201	PAH-145	CAS: 85-01-8	Phenanthrene
33.8997	1731.298	PAH-146	CAS: 120-12-7	Anthracene
36.1256	1795.8583	PAH-147	CAS: 7372-88-5	4-Methyldibenzothiophene
37.516	1837.1611	PAH-148	CAS: 832-71-3	3-Methylphenanthrene
37.6575	1841.4053	PAH-149	CAS: 2531-84-2	2-Methylphenanthrene
38.2247	1858.5016	PAH-150	CAS: 883-20-5	9-Methylphenanthrene
38.3798	1863.1972	PAH-102	CAS: 832-69-9	Phenanthrene, 1-methyl-
39.187	1887.8191	PAH-223	CAS: 89816-99-9	4-Ethyldibenzothiophene
41.2223	1951.1727	PAH-225	CAS: 1576-67-6	3,6-Dimethylphenanthrene
42.0701	1978.1	PAH-224	CAS: 132034-86-7	4-Propyldibenzothiophene
42.2671	1984.4004	PAH-226	CAS: 483-87-4	1,7-Dimethylphenanthrene
42.4458	1990.1356	PAH-151	CAS: 206-44-0	Fluoranthene
43.4188	2021.5995	PAH-227	CAS: 20291-72-9	1,2-Dimethylphenanthrene
43.8627	2036.0897	PAH-152	CAS: 129-00-0	Pyrene
45.6718	2096.1079	PAH-228	CAS: 66271-32-7	2,6,9-Trimethylphenanthrene
46.9396	2139.1454	PAH-229	CAS: 30436-55-6	1,2,6-Trimethylphenanthrene
47.0289	2142.2059	PAH-230	CAS: 146448-87-5	1,2,5-Trimethylphenanthrene
47.3486	2153.1969	PAH-231	CAS: 60490-68-8	1,2,7-Trimethylphenanthrene
50.6906	2271.1949	PAH-153	CAS: 204256-39-3	1,2,6,9-Tetramethylphenanthrene
53.1348	2361.1964	PAH-154	CAS: 56-55-3	Benzo[a]anthracene
53.3364	2368.7669	PAH-103	CAS: 218-01-9	Chrysene
57.3205	2523.1316	PAH-155	CAS: 3351-28-8	1-Methylchrysene
58.746	2580.5945	PAH-156	CAS: 2732-58-3	6-Ethylchrysene
60.6651	2659.9685	PAH-195	CAS: 6910-41-4	6-Propylchrysene
60.762	2664.0379	PAH-158	CAS: 205-99-2	Benzo[b]fluoranthene
60.9273	2670.9936	PAH-159	CAS: 207-08-9	Benzo[k]fluoranthene
62.322	2730.3874	PAH-237	CAS: 192-97-2	Benzo[e]pyrene
62.5857	2741.7588	PAH-160	CAS: 50-32-8	Benzo[a]pyrene
63.0926	2763.7424	PAH-171	CAS: 198-55-0	Perylene
69.2883	3046.4538	PAH-178	CAS: 193-39-5	Indeno[1,2,3-cd]pyrene
69.5594	3059.444	PAH-174	CAS: 53-70-3	Dibenz[a,h]anthracene
70.5059	3105.2069	PAH-179	CAS: 191-24-2	Benzo[ghi]perylene

