

Introduction to UV-Mutual Automated Peak Matching (UV-MAP): How to Optimize the Input Parameters

ACD/Method Development Suite AutoChrom Console
Version 10.00

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Introduction

Matching peaks from run to run is one of the most difficult parts of method development. ACD/Method Development Suite AutoChrom Console¹ offers a new automated peak matching algorithm for LC/UV data. This algorithm is based on spectral comparison for each component in the dataset after the main peaks are found and labeled.

This Technical Note will explain each option of the UV Peak Matching Tool in detail and give you some tips on how to find the right settings.

Peak Picking Parameters

Solvent Separation Threshold

This parameter is used to filter the peaks of the solvent that appear due to the formation of a solvent front, wave of pressure and column wash. When these peaks are filtered, they are not taken into account when labeling and matching procedures are being performed. The program compares the UV spectrum of the solvent with the UV spectrum of each found peak and applies a threshold. If their similarity is higher than the threshold, the peak will not be labeled. In other words, this threshold allows you to exclude from the results the peaks that have spectra similar to the spectrum of the solvent. Increasing this parameter leads to a greater number of found and therefore labeled peaks.

We recommend setting this parameter to **6** as a starting point. It means that the parameter is actually turned off as it is hard to find a peak that has a UV spectrum similar to the solvent spectra to such a great extent.

You can gradually decrease this value to exclude the solvent peaks and reach the minimum value of **0.1**. In this case, all the peaks having spectra with the smallest similarity to solvent spectra will be recognized as solvent peaks and rejected.

So, the higher the value, the greater number of peaks that will be found.

Concentration Threshold

This threshold allows you to exclude very small peaks from the results. Let's say the maximum peak in the chromatogram is 100%. Then all other peaks are calculated in respect to this maximum peak. If the peak is below this specified threshold, this peak will not be found and

therefore labeled. Increasing the value of this parameter leads to decreasing the number of found peaks.

To find the right value for this parameter, we recommend to experiment with one file first where all the peaks are pretty well resolved.

Note To be able to work with one specific file within the project, you can filter it in the Query for Signals subwindow or place it in a new group. In this case, clicking **Apply UV Peak Matching** will open the parameters of the Peak Picking section only.

Set this parameter to **0** as a starting point. It means that the option is turned off and all the possible peaks will be found. Then look closely at small peaks and estimate their concentration (10%, 1%, 0.1%, 0.01%, and so on). Set the new estimated value and run it to see the results. All the peaks with a concentration below this threshold will be filtered from the output. If one of the desired peaks disappeared, decrease the value of this threshold. If you see more peaks than needed, increase the value of this parameter.

The greater the concentration threshold value, the fewer peaks that will be found.

In version 11.0, the components table will have one additional column – Concentration. The values in this column will help user to determine the value for Concentration Threshold parameter. If the concentration values for undesired peaks are small and for desired – big, then as threshold you can take any concentration value in the middle. If the concentration pattern is not consistent, this parameter is of no use.

Peak Quality

This is the main parameter that controls the process. In some cases, it is enough to adjust this parameter only.

The term of Peak Quality covers various characteristics of a peak—size, UV spectrum of the peak having a high intensity maximum, signal/noise ratio for the spectrum of the peak, as well as the peak purity. If the peak is big and well separated from the other peaks, and its UV spectrum has a few pure maxima, this peak has a very high quality.

If you set this parameter to its maximum value (**6**), the very good peaks only—peaks with high signal/noise ratio—will remain.

Decreasing this value to the minimal value (**2**) may result in extraneous peaks along with the good ones.

As practice shows, in most cases we operate with the values between 3 and 4. We recommend the following workflow:

1. Locate one file by applying filters it in the Query subwindow or placing it into a new group.
2. Click **UV Peak Matching Tool**.
3. Set 3 for **Peak Quality**.
4. Switch off all other thresholds (**Solvent Separation** to 6 and **Concentration** to 0.1).
5. Click **OK** and check how many peaks you have labeled.
6. Gradually increase the value of this parameter and find the maximum when all the needed peaks are found.
7. You may see that there a few undesired components are found. Then you need to use other thresholds (**Solvent Separation** and **Concentration**) to filter them out.

Estimated Peak Width

This parameter indicates the order of the peak width—its influence on the process can be insignificant in some cases.

The algorithm finds the components not on the top of the peak but in the leading and tailing slopes. It uses Δ time between two neighbor components. If this difference in time is less than the value of the **Estimated Peak Width** parameter and the components have similar spectra, the program combines them into one. If Δ time is greater than the value of this parameter, then we deal with two different components.

We recommend starting with a value **0.1**.

If you expect to see one component and the program gives you two in one peak, then you need to increase the value to 0.2.

If two narrow peaks with similar spectra are sitting too close to each other, the program may recognize one component instead of two. In this case, you need to decrease the value to 0.05. You can observe this situation when working with UPLC data where the peaks are very narrow.

Reprocess Already Processed Signals

If this option is checked, all selected signals will be processed. The previously processed data will be reprocessed with new settings.

If this option is not checked, the program processes only new data files and then it applies matching options to all signals. Sometimes you need to process datasets with different peak picking settings and then apply matching and this check box helps you to do so.

Discard Peaks Not Picked in Subsidiary Chromatograms

A subsidiary chromatogram is a single-wavelength chromatogram. In other words, an LC/DAD data set may be collected on one channel, and a second channel may record the 254 nm absorbance chromatogram.

If the **Discard Peaks Not Picked in Subsidiary Chromatograms** option is selected, the UV-MAP function will only return peaks that have been picked in the associated single wavelength chromatogram(s).

Preserve Actually Assigned Peaks

If this option is checked, previously processed datasets will keep their peak names and retention time values.

Search Only Previously Assigned Peaks

You may wish to run UV-MAP using data files that have been previously processed, either manually, or with UV-MAP.

If the **Search Only Previously Assigned Peaks** option is selected, UV-MAP will be applied only to peaks that have been picked during previous data processing.

Peak Matching Parameters

Spectral Matching Accuracy

This parameter helps us to compare UV spectra. If the similarity coefficient is less than the value of this parameter, spectra are different. If it is greater than the value of Spectral Matching Accuracy, spectra are equal.

Set the value to **0.8** as a starting point. As practice shows, it rarely needs to be changed.

If you see that the same component receives different names in different injections, you should decrease this value. If you see that two different components receive the same name, you should increase this value.

Overload Signal Threshold %

This is a very straightforward parameter that helps to deal with big and small peaks separately.

A sample may contain one or two compounds with relatively high concentration and impurities with much lower concentration. Due to high concentration, a main peak is overloaded, its spectrum changes while moving from leading area to the top, or from the top to the tailing area of the peak. To separate a main peak from impurities, we need to properly compare their spectra.

The **Overload Signal Threshold** parameter divides all the components in each injection into two groups. One group contains big or overloaded peaks and has a concentration greater than the barrier we set. Another group contains peaks with a concentration less than this barrier. Then the matching process starts. The algorithm in this case compares spectra within each group separately.

We recommend starting with a value of **100%**. It means that the parameter is switched off. Then take a look at the dataset. If you notice one or a few very big peaks and many really small ones, you definitely need to use this parameter. This value (**Overload Signal Threshold %**) should be higher than the concentration of impurities and lower than the concentration of the main component. As a result, impurities and the main component will be matched independently.

In the Experiments table, the program shows the component names (C_1, C_2, ...). If you use this parameter, as a result the small peaks are named (numbered) first and overloaded peaks have the biggest numbers.

Use Concentration Ratio

If you inject the same sample under different conditions, it means that in different experiments you are dealing with the same concentration for each compound. In this case, turn on this parameter and the program will take into account the concentration ratio in addition to spectral similarity.

We recommend using a value of **0.1** in both ratio boxes as a starting point. You will rarely need to change it to greater value. The program will compare the concentration of the components first. If concentrations differ greater than 10 times, you have different components. If the difference is less than 10 times, the program compares UV spectra and makes a decision.

Example

Prepare the files

For this example, the uv_peak_matching.ZIP file will be utilized. We will deal with a mixture of food coloring compounds with quite different spectra and similar concentration.

1. Download the uv_peak_matching.ZIP file and save it somewhere.
2. Extract all the files and folders contained in the uv_peak_matching.ZIP file.
3. As a result, you will see an EXAMPLE folder that contains the whole project: a FOOD_ADD.AWX file and a folder with the same name (FOOD_ADD).
4. Open this project in the Task window of the AutoChrom Console.
5. The processing will be performed for the signals in Wave 2.

Waves			
#	Wave	Status	Attribute
1	Column	Complete	
2	Gradient & Temperat...	Complete	Hypersil GOLD

Note All the derived signals are manually processed and the peaks are named. We are going to apply the UV Peak Matching tool and extract the same main components.

Experiments												
Experiment	Status	Suit.	Suit. ^{***}	Total	E102...	benzoic acid	sorbic acid	E132...	E12...	E11...	E12...	E13...
25°C / 0-80% (11 min)	Complete	0.07	0.163	8/8	2.9	3.13	3.5	3.38	3.94	4.23	5.49	7.07
Sample	-	0.07	0.163	8/8	2.9	3.13	3.5	3.38	3.94	4.23	5.49	7.07
Trial #1	Complete	0.07	0.163	8/8	2.9	3.13	3.5	3.38	3.94	4.23	5.49	7.07
UV dad1A.ch	Complete	-	-	0/8								
UV dad1.uv	Complete	-	-	0/8								
UV dad1.uv	-	0.07	0.163	8/8	2.9	3.13	3.5	3.38	3.94	4.23	5.49	7.07
25°C / 2.8-35.4% (11 min)	Complete	0.207	0.286	8/8	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94
Sample	-	0.207	0.286	8/8	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94
Trial #1	Complete	0.207	0.286	8/8	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94
UV dad1A.ch	Complete	-	-	0/8								
UV dad1.uv	Complete	-	-	0/8								
UV dad1.uv	-	0.207	0.286	8/8	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94
25°C / 0-35.6% (10 min)	Complete	0	0.057	8/8	3.3	3.34	3.88	4.19	5.26	5.8	8.32	11.3
Sample	-	0	0.057	8/8	3.3	3.34	3.88	4.19	5.26	5.8	8.32	11.3
Trial #1	Complete	0	0.057	8/8	3.3	3.34	3.88	4.19	5.26	5.8	8.32	11.3
UV dad1A.ch	Complete	-	-	0/8								
UV dad1.uv	Complete	-	-	0/8								
UV dad1.uv	-	0	0.057	8/8	3.3	3.34	3.88	4.19	5.26	5.8	8.32	11.3
25°C / 0-3% (0.42 min); 3-32.2% ...	Complete	0.572	0.615	8/8	2.97	3.18	3.72	4	5.38	6.04	9.34	13.18
Sample	-	0.572	0.615	8/8	2.97	3.18	3.72	4	5.38	6.04	9.34	13.18
Trial #1	Complete	0.572	0.615	8/8	2.97	3.18	3.72	4	5.38	6.04	9.34	13.18
UV dad1A.ch	Complete	-	-	0/8								
UV dad1.uv	Complete	-	-	0/8								
UV dad1.uv	-	0.572	0.615	8/8	2.97	3.18	3.72	4	5.38	6.04	9.34	13.18

6. In the Query for Signals subwindow, while holding CTRL, select **Selected Wave**, and **Spectral Data**.

Query for Signals			Signals	
NOT	Query	Qty		
	All Signals	21		dad1.uv
AND	Selected Wave	15		dad1.uv
OR	Selected Signals	0		dad1.uv
	Chromatogram	14		dad1.uv
	Spectral Data	7		dad1.uv
	Processed Data	5		
	Table of Peaks	12		
	Luna C18(2)	3		
	Hypersil GOLD	15		
	Zorbax Eclipse XDB-C8	3		

7. Click **UV Peak Matching** .

8. Set the following options.

UV Peak Matching Settings

Peak Picking

Solvent Separation Threshold: 6

Concentration Threshold: 0 %

Peak Quality Threshold: 3

Estimated Peak Width: 0.1 min

Reprocess Already Processed Signals

Discard Peaks not Picked in Subsidiary Chromatograms

Preserve Previously Assigned Peaks

Search Only Previously Determined Components

Peak Matching

Spectral Matching Accuracy: 0.8

Overload Signal Threshold: 100 %

Use Concentration Ratio

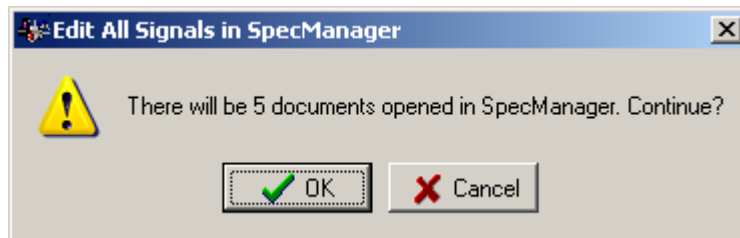
Minimal Ratio: 0.1

Maximal Ratio: 0.1

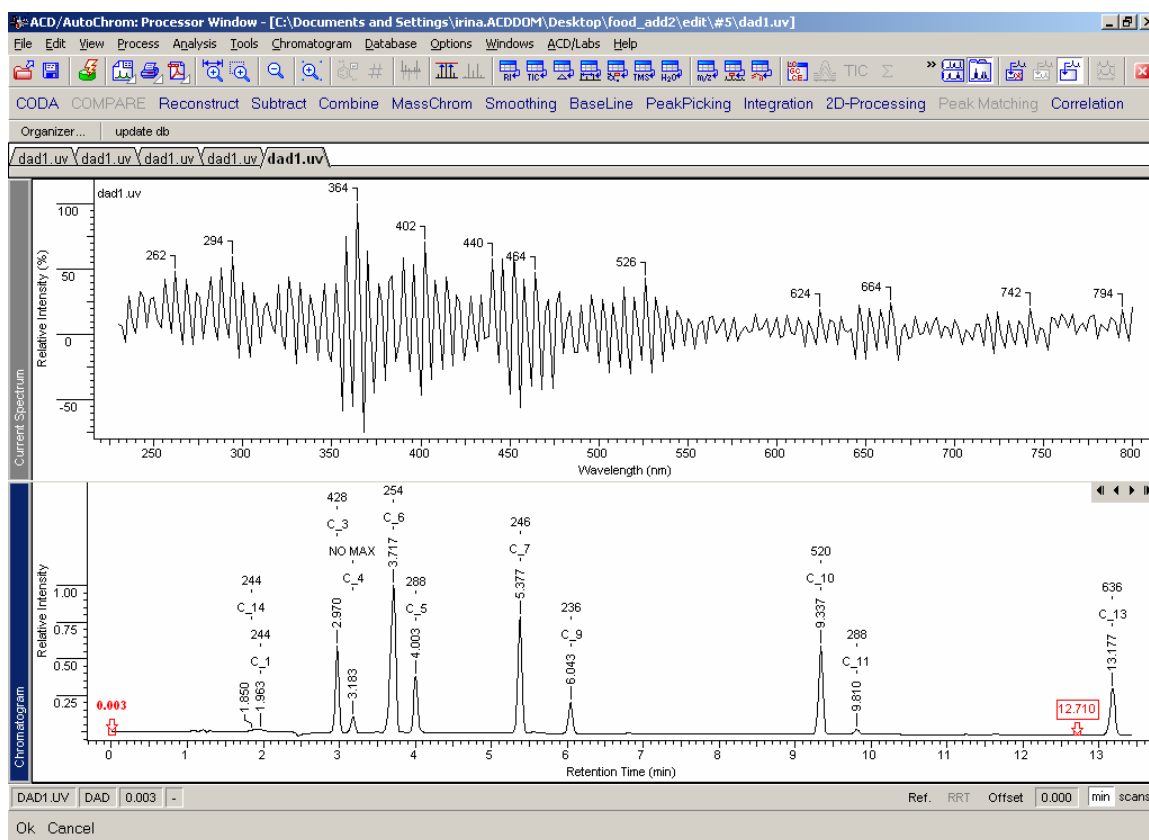
OK Cancel Help

Note The values we started with were recommended in the sections above. Remember, that we manipulate with the **Peak Quality Threshold** parameter first. The **Use Concentration** check box is selected as we are dealing with the same sample and concentration through the whole project.

- Click **OK** to start the process.
- As a result, you will see 15 new components added to the end of the Experiments table. Take a close look at the found peaks and their retention times. You should notice that some peaks have similar retention times with the peaks that were processed manually. The algorithm extracted more peaks than needed. We will try to eliminate the number of found components by changing the settings.
- In the upper right subwindow, select the **Chromatogram** tab to see all the extracted components and labeled peaks. Keep in mind that you are looking at the restructured, composite chromatogram.
- In the Query for Signals subwindow, you should still have **Selected Wave** and **Spectral Data** highlighted.
- In the Signals subwindow, right-click anywhere and choose **Edit in SpecManager (All)**.
- Click **OK** on the message shown below.



- The program will switch you to the Processor window and open all the signals in separate windows.



At this point you see the real data. You also see the result of processing that was performed in the Task window. Notice a lot of unnecessary labeled peaks in the Chromatogram Pane.

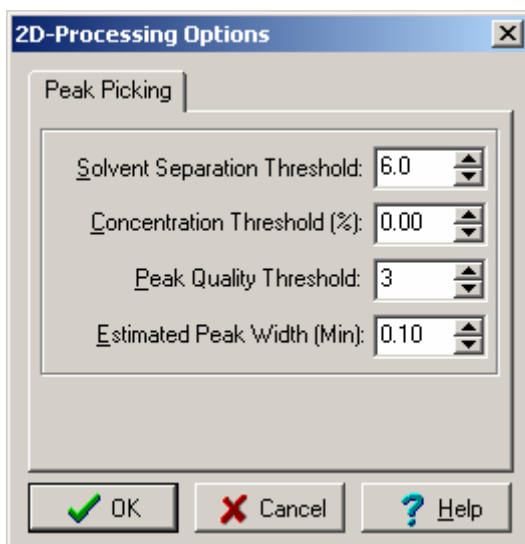
Within the Processor window of SpecManager we are going to improve the peak picking settings, so only main peaks are labeled, and apply them later in the Task window for all signals. As a result you will see less than 15 extracted components.

1. Navigate to the first file and on the Operation toolbar, click **2D-Processing Mode**
2D-Processing.

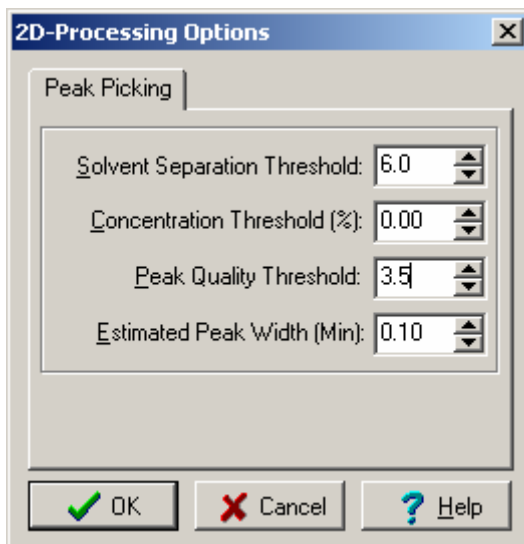
2. Click **OK** on the message that appears.
3. On the top toolbar click **Options**.



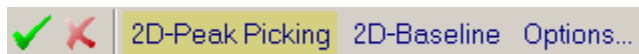
4. You will see six Peak Picking options that you saw when using the UV Peak Matching tool. As you remember, we started with the following settings and had a lot of undesired peaks.



- Let's increase the value of Peak Quality Threshold and see the results.
- In the **2D-Processing Options** dialog box, change the Peak Quality Threshold to 3.5 and click **OK**.

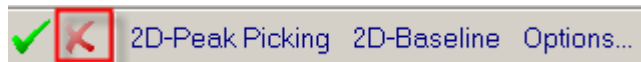


- To apply new settings, click **Pick 2D-Peaks**.



- The result is a bit better, but there are still a couple of very small peaks which we are not interested in (peaks at 1.938, 4.058, and 6.472 min).
- In the **2D-Processing Options** dialog box, change the **Peak Quality Threshold** to 4 and apply. The result is much better.
- We improved the settings for the first file; let's see how other signals are processed when the same settings are applied.

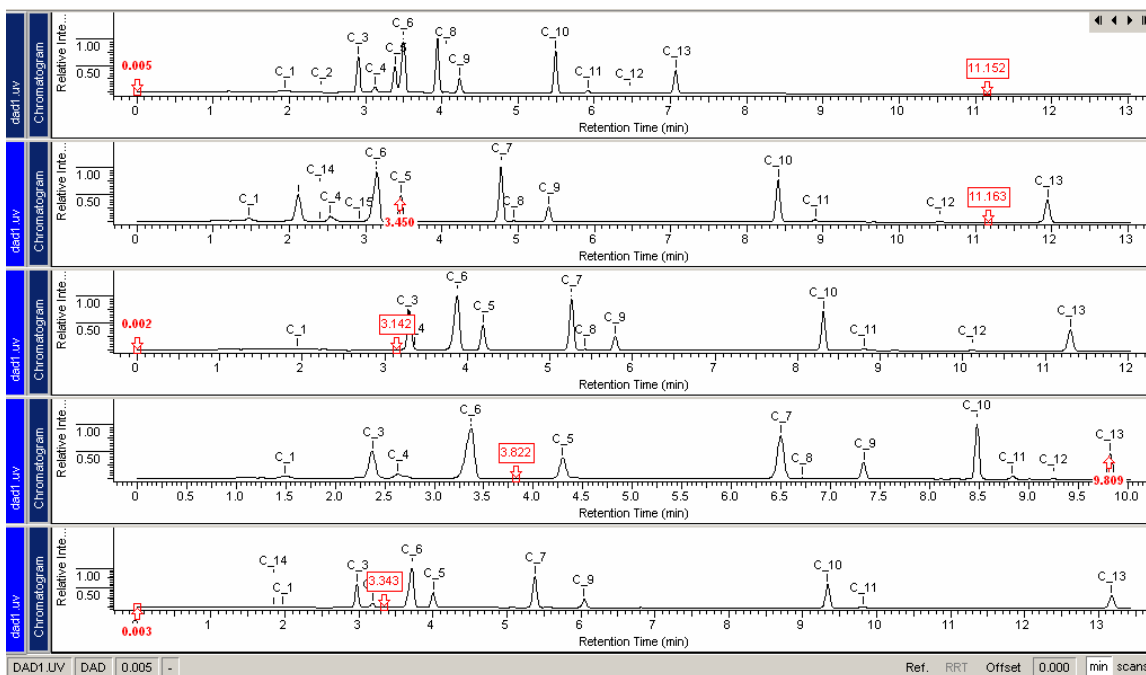
11. Cancel all the changes by clicking **Cancel Operation**.



12. From the **Windows** menu, choose **Tile** to see all the data files at once.

13. From the **Windows** menu, choose **Select All**.

14. On the **View** menu, point to **Panes Display**, and then choose **Chromatogram Pane only**. This will allow you to look at the chromatograms closer.



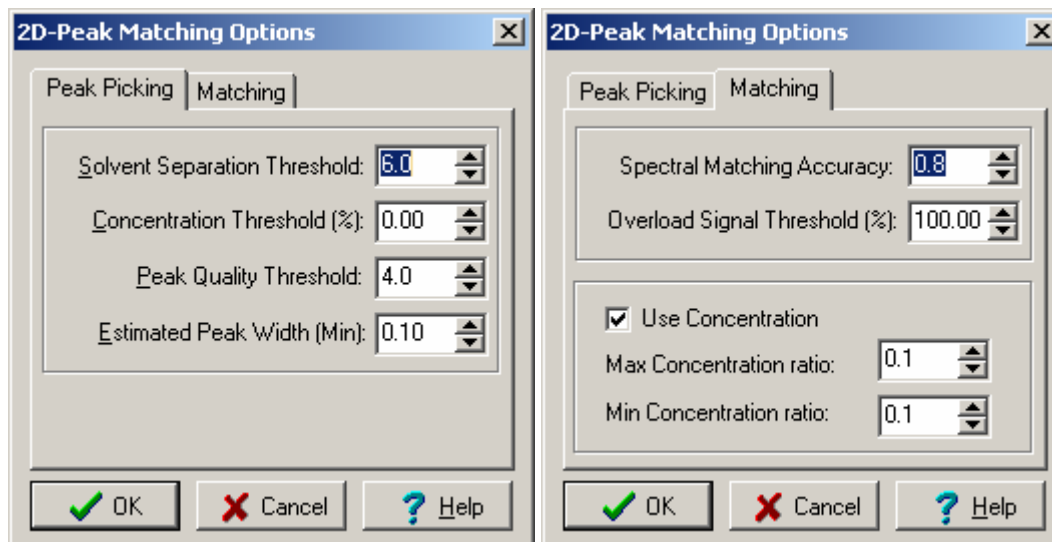
Peak Matching

15. Enter the Peak Matching mode by clicking

16. Click **OK** on the message that appears.

17. On the Operation toolbar click **Options**.

18. The options for Peak Picking should be kept the same after we made changes working with the first signal. On the **Matching** tab, specify settings as shown below.

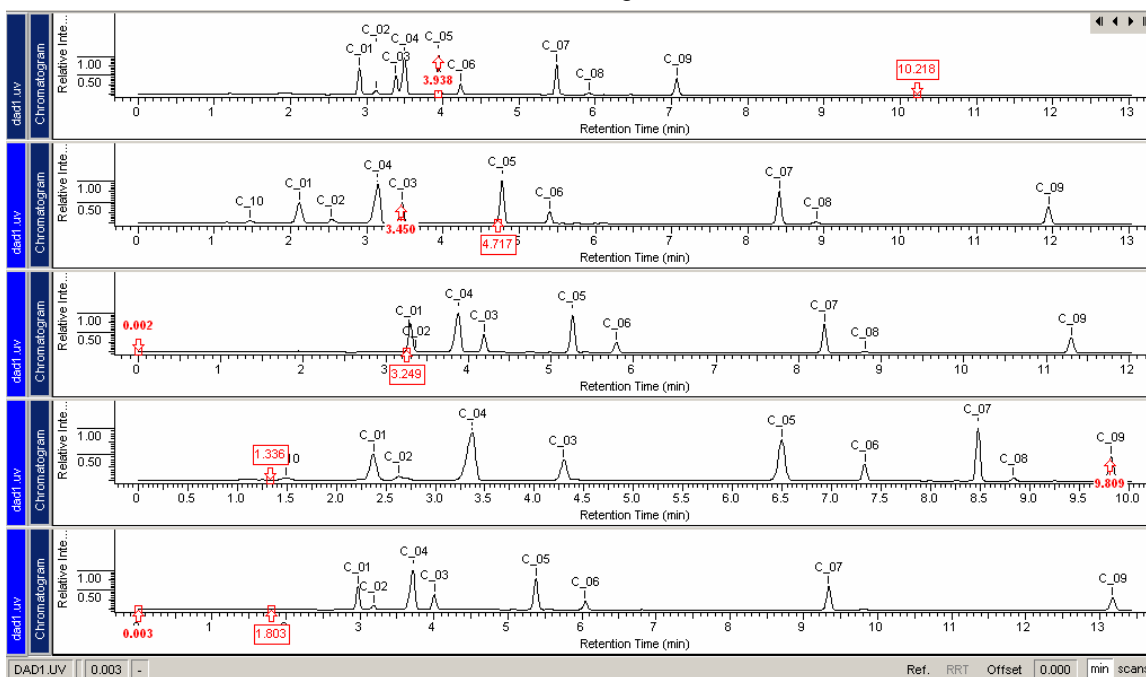


19. Click **OK** to accept these options.

20. Click **Run** to start processing.

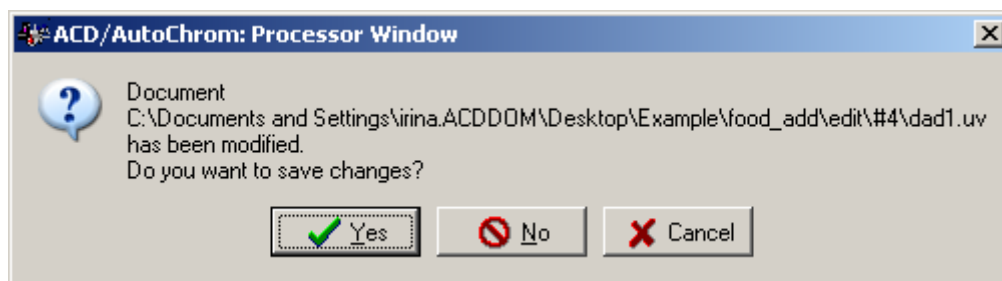


21. Observe the result. It seems that the new settings work.



22. Remember the last settings and click **Cancel** on the bottom.

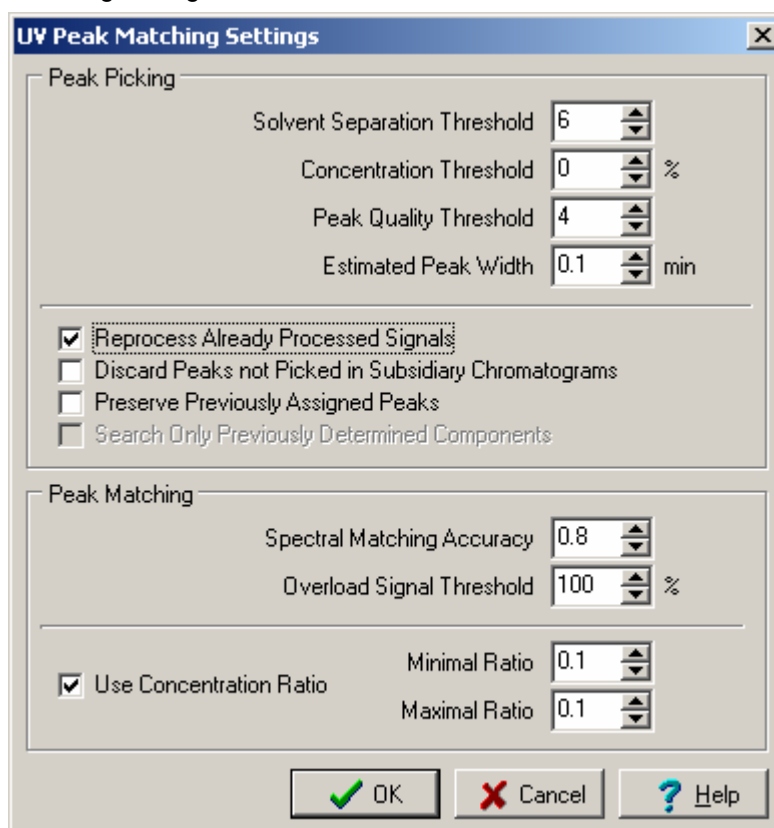
23. Click **No** on the message that appears (see below).



24. You are now in the Task window.

25. Click **UV Peak Matching**  again.

26. Specify the following settings and click **OK**.




You should see 10 additional components instead of 15.

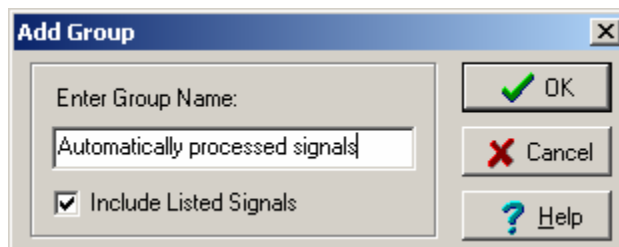
We can now reconcile the peaks that were processed manually (first 8 columns in the Experiments table) with the components that were extracted with the help of the UV Peak Matching tool. To do that, we need to create two groups and then reconcile.

The Query for Signals and Signals subwindows should still look similar to this:







Query for Signals		Signals	
NOT	Query	Qty	
	All Signals	21	dad1.uv
AND	Selected Wave	15	dad1.uv
OR	Selected Signals	0	dad1.uv
	Chromatogram	14	dad1.uv
	Spectral Data	7	dad1.uv
	Processed Data	5	
	Table of Peaks	12	
	Luna C18(2)	3	
	Hypersil GOLD	15	
	Zorbax Eclipse XDB-C8	3	

You are ready to create the first group that will include all filtered signals.

1. Click **Add Group** .
2. In the **Add Group** dialog box that appears, specify the **Group Name**, select the **Include Listed Signals** check box, and click **OK**.



3. In the Query for Signals subwindow, you see that a new group was created.

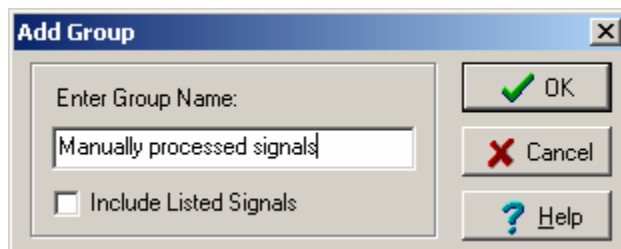
Query for Signals			Signals	
NOT	Query	Qty		
	All Signals	21		dad1.uv
AND	Selected Wave	15		dad1.uv
OR	Selected Signals	0		dad1.uv
	Chromatogram	14		dad1.uv
	Spectral Data	7		dad1.uv
	Processed Data	5		
	Table of Peaks	12		
	Luna C18(2)	3		
	Hypersil GOLD	15		
	Zorbax Eclipse XDB-C8	3		
	Automatically processed ...	5		

4. In the Query for Signals subwindow, highlight the Selected Wave line.

Query for Signals		
NOT	Query	Qty
	All Signals	21
AND	Selected Wave	15
OR	Selected Signals	0
	Chromatogram	14
	Spectral Data	7
	Luna C18(2)	3
	Hypersil GOLD	15
	Zorbax Eclipse XDB-C8	3
	pp	1
	Automatically processed signals	5

5. Click **Add Group** .

6. In the **Add Group** dialog box that appears, specify the **Group Name**, clear the **Include Listed Signals** check box, and click **OK**.



7. Notice that this time the new group does not have any signals included. It can be observed in the Signals subwindow.

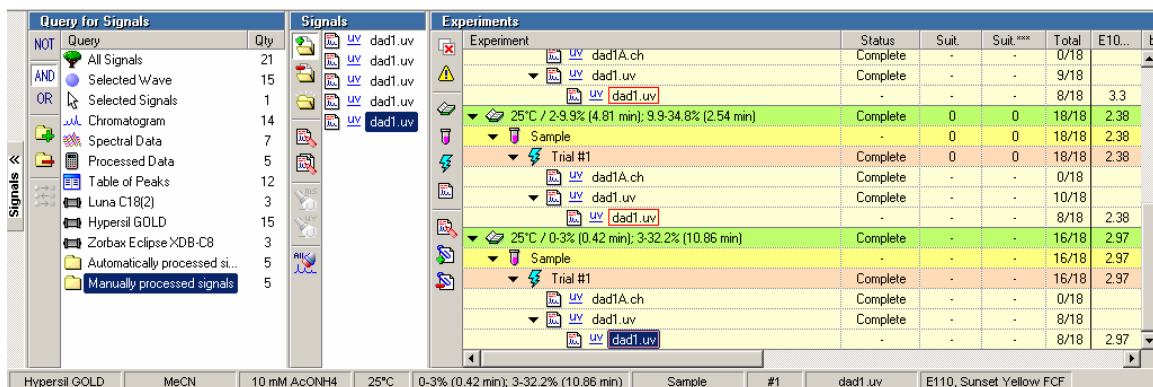
Query for Signals			Signals
	Query	Qty	
NOT	All Signals	21	
AND	Selected Wave	15	
OR	Selected Signals	0	
	Chromatogram	14	
	Spectral Data	7	
	Processed Data	5	
	Table of Peaks	12	
	Luna C18(2)	3	
	Hypersil GOLD	15	
	Zorbax Eclipse XDB-C8	3	
	Automatically processed signals	5	
	Manually processed signals	0	

When any new group is created it is allowed to add more signals to this group. You can see that the button shown below is highlighted. If not, you can select it manually.




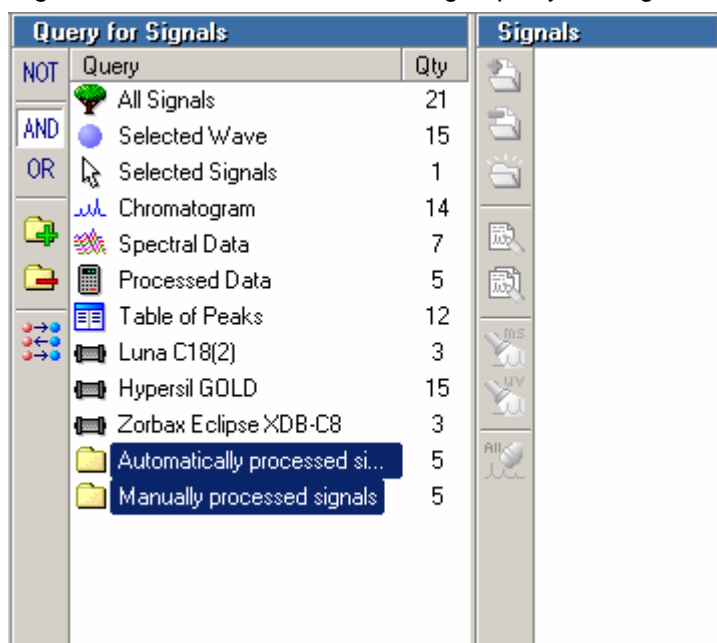
We are going to add all the signals that were manually processed to this group.

1. In the Experiments table carefully click on the lines with derived signals. Every click will add the signal to the group.



Query	Qty	Signal	Experiment	Status	Suit.	Suit***	Total	E10...	bc
All Signals	21	dad1.uv	dad1A.ch	Complete	-	-	0/18		
Selected Wave	15	dad1.uv	dad1.uv	Complete	-	-	9/18		
Selected Signals	1	dad1.uv	dad1.uv	-	-	-	8/18	3.3	
Chromatogram	14	dad1.uv	25°C / 2-9.9% (4.81 min); 9.9-34.8% (2.54 min)	Complete	0	0	18/18	2.38	
Spectral Data	7	dad1.uv	Sample	-	0	0	18/18	2.38	
Processed Data	5	dad1.uv	Trial #1	Complete	0	0	18/18	2.38	
Table of Peaks	12	dad1.uv	dad1A.ch	Complete	-	-	0/18		
Luna C18(2)	3	dad1.uv	dad1.uv	Complete	-	-	10/18		
Hypersil GOLD	15	dad1.uv	dad1.uv	-	-	-	8/18	2.38	
Zorbax Eclipse XDB-C8	3	dad1.uv	25°C / 0-3% (0.42 min); 3-32.2% (10.86 min)	Complete	-	-	16/18	2.97	
Automatically processed si...	5	dad1.uv	Sample	-	-	-	16/18	2.97	
Manually processed signals	5	dad1.uv	Trial #1	Complete	-	-	16/18	2.97	
		dad1A.ch	dad1A.ch	Complete	-	-	0/18		
		dad1.uv	dad1.uv	Complete	-	-	8/18		
		dad1.uv	dad1.uv	-	-	-	8/18	2.97	

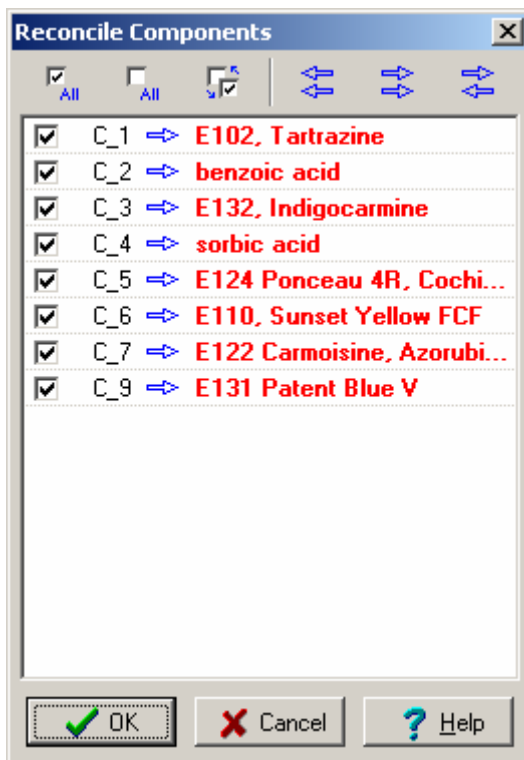
2. Click this button  again to disable it.
3. In the Query for Signals subwindow, select both new groups by holding CTRL.



Query	Qty
All Signals	21
Selected Wave	15
Selected Signals	1
Chromatogram	14
Spectral Data	7
Processed Data	5
Table of Peaks	12
Luna C18(2)	3
Hypersil GOLD	15
Zorbax Eclipse XDB-C8	3
Automatically processed signals	5
Manually processed signals	5

4. Click **Reconcile Components**  on the left.

- A new window appears that shows you what peaks are to be reconciled and what peak names to leave.



- Click **OK** and the Experiments table is now cleaner.

Experiments		Status	Suit.	Suit. ^{***}	Total	E10...	benz...	sorbi...	E13...	E12...	E11...	E12...	E13...	C_8	C_10
Experiment	25°C / 0-80% (11 min)	Complete	-	-	9/10	2.9	3.13	3.5	3.38	3.94	4.23	5.49	7.07	5.92	
Sample		-	-	-	9/10	2.9	3.13	3.5	3.38	3.94	4.23	5.49	7.07	5.92	
Trial #1		Complete	-	-	9/10	2.9	3.13	3.5	3.38	3.94	4.23	5.49	7.07	5.92	
UV	dad1A.ch	Complete	-	-	0/10										
UV	dad1.uv	Complete	-	-	9/10	2.91	3.13	3.5	3.38	3.94	4.23	5.49	7.07	5.92	
UV	dad1.uv	-	-	-	8/10	2.9	3.13	3.5	3.38	3.94	4.23	5.49	7.07	5.92	
Experiment	25°C / 2.8-35.4% (11 min)	Complete	0	0.022	10/10	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94	8.9	1.47
Sample		-	0	0.022	10/10	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94	8.9	1.47
Trial #1		Complete	0	0.022	10/10	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94	8.9	1.47
UV	dad1A.ch	Complete	-	-	0/10										
UV	dad1.uv	Complete	0	0.022	10/10	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94	8.9	1.47
UV	dad1.uv	-	-	-	8/10	2.12	2.54	3.14	3.46	4.78	5.4	8.41	11.94	8.9	1.47
Experiment	25°C / 0-35.6% (10 min)	Complete	-	-	9/10	3.29	3.35	3.88	4.19	5.26	5.8	8.32	11.3	8.8	
Sample		-	-	-	9/10	3.29	3.35	3.88	4.19	5.26	5.8	8.32	11.3	8.8	
Trial #1		Complete	-	-	9/10	3.29	3.35	3.88	4.19	5.26	5.8	8.32	11.3	8.8	
UV	dad1A.ch	Complete	-	-	0/10										

Hypersil GOLD MeCN 10 mM AcONH4 25°C 0-3% (0.42 min); 3-32.2% (10.86 min) Sample #1 dad1.uv E110, Sunset Yellow FCF

Conclusion

This example showed how to adjust the peak picking parameters in order to optimize the UV-MAP peak-matching algorithm. We have seen that the most important parameter to optimize is the **Peak Quality** parameter.

Appendix A:

Summary of UV-MAP Starting Parameters

Parameter	Start Value	Range	Comments
Peak Picking Parameters			
Solvent Separation Threshold	6 (off)	0.1–6	Use this parameter to exclude solvent peaks from peak picking
Concentration Threshold	0 (off)	0–100% of largest peak in chromatogram	Use this parameter to exclude low-intensity peaks from peak picking
Peak Quality	3	2–6	Main parameter to adjust Increase if too many minor peaks are picked Decrease if peaks are missed during peak picking
Estimated peak width	0.1		May need to decrease value if two discernible, closely eluting peaks are selected by the algorithm as one peak; may need to increase value if one large peak is marked as two peaks by the algorithm
Reprocess Already Processed Signals	On		
Discard Peaks not Picked in Subsidiary Chromatograms	Off		
Preserve Actually Assigned Peaks	Off		
Search Only Previously Assigned Peaks	Off		
Peak Matching Parameters			These parameters rarely need to be changed
Spectral Matching Accuracy	0.8		
Overload Signal Threshold %	100% (off)		
Use Concentration Ratio	If On, use 0.1, 0.1		

References

1. ACD/Method Development Suite. www.acdlabs.com/meth_develop/ Advanced Chemistry Development, Inc., Toronto, ON, Canada. 11 September, 2007.