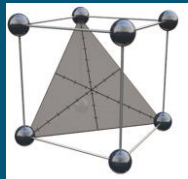
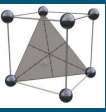


# FUSION QBD<sup>®</sup> - PEAK TRACKING

## Best Practice Guide



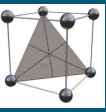


## Provision of Good Data:

**! High quality data in terms of peak integration and spectral information is the key for accurate peak tracking.**

## Increase the signal:

- Do not prepare the sample with impurities, related substances, and degradants at standard QC target amounts (e.g., 0.50% of API concentration), as this will artificially reduce the peak signals.
- Conduct an initial sample workup study to identify the optimum injection volume and sample concentration to maximize the UV and MS signal without significantly increasing the peak baseline widths.
- Consider using MaxPlot in initial screening studies when critical sample compounds have very low signal.
- Select a 2D UV channel which minimizes baseline noise.
- Adjust peak start and peak end points to minimize peak widths by eliminating incorrectly included baseline.
- Consider using runs with different injection volumes from the same sample solution in and FDS experiment, runs with lower injection volume to track large peaks, runs with higher injections volume to track small peaks, avoid integrating peaks that are not of interest in the respective run.
- Spike your sample, if sample material is available

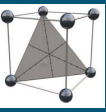


## Provision of Good Data:

**! High quality data in terms of peak integration and spectral information is critical for accurate peak tracking.**

## Peak Integration

- Make sure to integrate all peaks of interest (that should be included during peak tracking and data modelling) in the UV channel in each experiment chromatogram. This includes manual corrections such as using the “drop-to-baseline” function to add visible but partially coeluted peaks such as shoulder peaks.
- Visually map the integration in the TIC against the UV chromatograms. Set the MS smoothing parameters to obtain reasonably accurate retention times for the MS peaks. Each peak in the UV channel should have a corresponding TIC channel peak. Exceptions: Peaks that either do not ionize or have no UV absorbance.
- Do not integrate peaks which should be excluded. For example, you should consistently delete the following peaks from the peak table in each experiment chromatogram:
  - Early or late eluting solvent or matrix peaks which in no case interact with any of the peaks of real interest.
  - Incorrectly integrated baseline noise peaks.

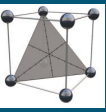


## Provision of Good Data:

**! High quality data in terms of peak integration and spectral information is critical for accurate peak tracking.**

### Critical

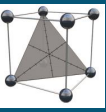
- Small Peaks won't provide enough quality of spectral information and are therefore difficult to track.
- Peaks with identical MS traces or PDA spectra are normally only identifiable during tracking based on traditional peak results such as area, retention, width, and height.
- Poor chromatographic quality, such as carry over effects, peak splitting, and high noise will significantly compromise peak tracking and therefore the resulting accuracy of the data models.
- Basically, automated peak tracking requires spectral information, that is unique for every component, and can therefore clearly characterize a peak component. If this is not possible, in case of isomers, there are still two options left, if those compounds are available as a pure reference material:
  1. Prepare a separate sample solution for such an isomere. You can define in Fusion QbD, that a certain compound should only be tracked in a specified degradation path chromatogram.
  2. Prepare an artificial sample solution, where you made sure, that for each compound a significantly different peak area value is obtained, that is characteristic for each compound.



## Peak Tracking – Recommended Workflow after Data Import

### Global Tracking Method (GTM) for automated Peak Tracking

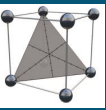
- 1) The GTM can be considered as the tracking reference and contains MS traces, UV spectra traces, and traditional peak results (areas, retention times, etc.). Entries in the UV data table will only be set, if MS data are absent or should be ignored (Either traditional or spectral data in addition, if imported).
- 2) UV spectra data should only be imported when either MS data are not available or when one or more peaks do not ionize (no MS signal).
- 3) The GTM also defines a reference component which supports tracking. By default this is the largest peak in the chromatogram used to generate the GTM. In small molecule studies this will normally be the API.
- 4) The GTM should include each component that should be included in the tracking.



## Peak Tracking – Recommended Workflow after Data Import

### Creation of the Global Tracking Method (GTM)

- 1) The initial creation of the GTM should be based on a run with the highest number of peaks. (Check Run Table in Data Review mode, No of Peaks column)
- 2) When one or more peaks are missing from the best available run chromatogram (e.g., due to coelution), then you should add the “missing” peak to the generated GTM using a chromatogram in which the missing peak is maximally resolved from all other peaks. (Performing a first tracking with the GTM might facilitate a first search for missing peaks).
- 3) Such effects can be identified by comparing area values and spectral information from each run against the GTM values.
- 4) When you are sure, that the GTM does not contain data from coelution effects anymore, you can run the final tracking, Fusion should now also detect the additional compounds, where they are coeluting.



## Peak Tracking – Recommended Workflow after Data Import

### Example of how to detect and correct a coelution in the initial GTM:

From the tracking an API peak with an area value much lower than identified in the reference run for the GTM is detected.

	Name	RT (min)	Area	Base
1	API	1.468	1,206,893	
2	A	1.666	33,882	
3	B	1.664		
4	D	1.666		
5	O	1.741	70,882	
6	J	1.741		
7	C	1.741		
8	E	2.170	10,919	
9	F	2.170		
10	H	2.664	81,751	
11	T	2.838	53,352	

Run #	Component Name	RT (min)	Area	Expected Mass 1	E
1	API	5.698	2,728,759	482.2	
2	B	6.070	101,067	480.2	
3	C	6.264	168,017	540.2	
4	D	6.717	26,083	99.9	
5	E	6.795	2,027	100.4	
6	F	7.068	2,432	99.9	
7	G	7.157	14,467	100.1	

Correction of the API entry: The initial API entry (from run 49 in the example) needs to be deleted in the GTM and a new one has to be created from the run where this coelution does not appear (run 7 in this example).

Additions to the GTM: The peaks from run 7 that are coeluting in run 49 need to be added to the GTM

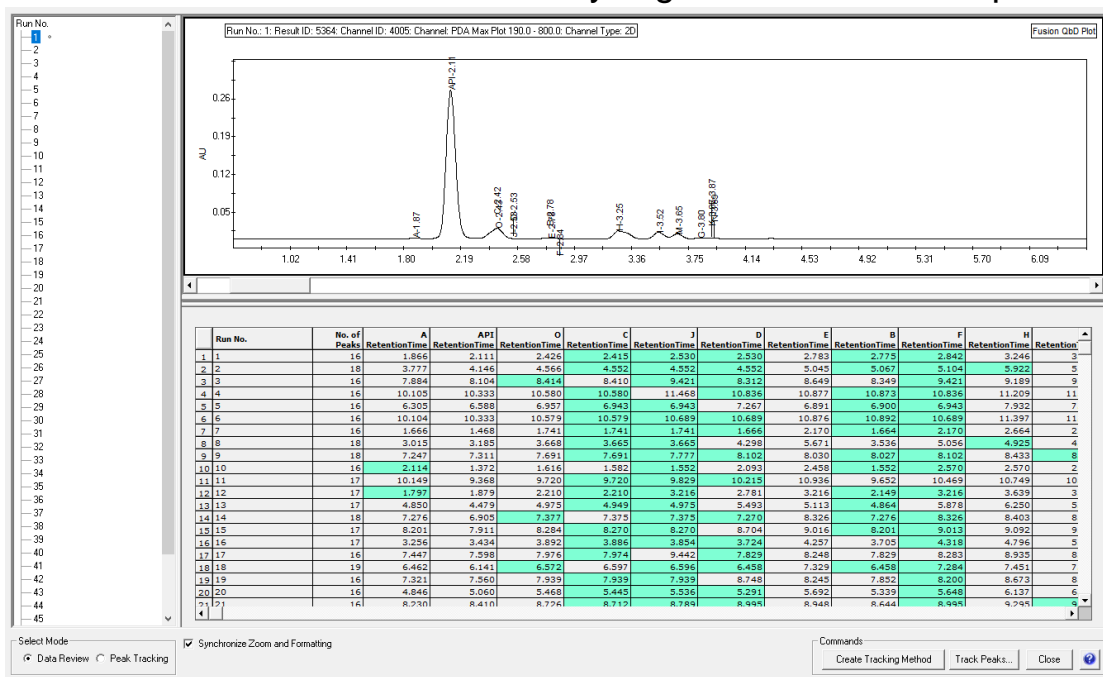


## Peak Tracking – Recommended Workflow after Data Import

### Data Review Mode

After the final tracking from the GTM switch to the Data Review mode.

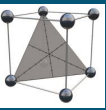
Here an overview of the entire study is given. For each component an entry is required.



Entries highlighted in GREEN stand for components that have been identified as a coelution.

The Review Mode also supports the check for consistency and plausibility.

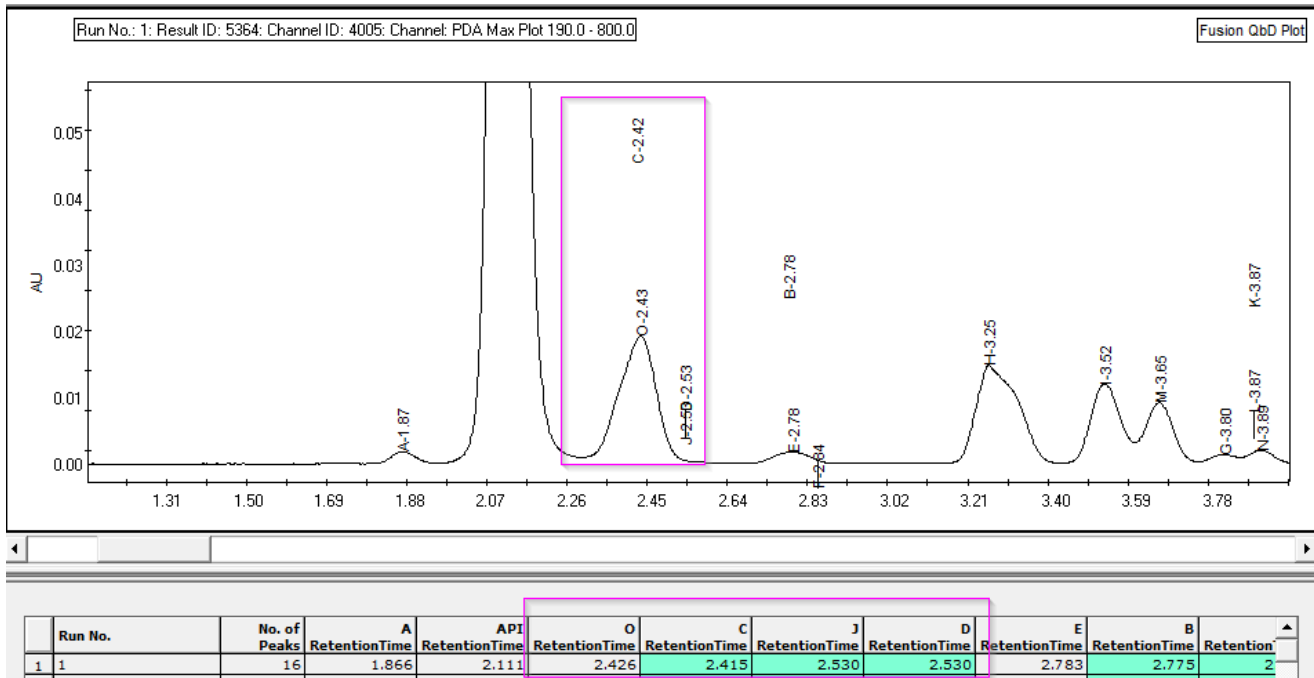


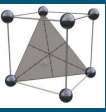


## Peak Tracking – Recommended Workflow after Data Import

### Data Review Mode

In the example component O has been detected and component C, J and D are identified as coelution





## Peak Tracking – Recommended Workflow after Data Import

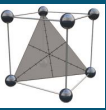
### Manual Adjustments of Component Assignment

When automated peak tracking is completed you should review each individual run regarding the following aspects:

- Are there still unnamed peaks?
- Is each component name from the GTM used to assign a peak?
- Is the assignment plausible? Check against peak areas against areas entered in the GTM. Check peak shape, is there an indication for an coelution that hasn't been identified?
- Is the elution order aligned with your expectation\* and can any misassignments be excluded?

To correct for the above-mentioned scenarios the PeakTracker offers several commands for manual interventions that can be launched from the context menu in the Peak Table.

\* Be carefully check traditional peak data an spectral information against the GTM, of course changes in elution can and will occur!



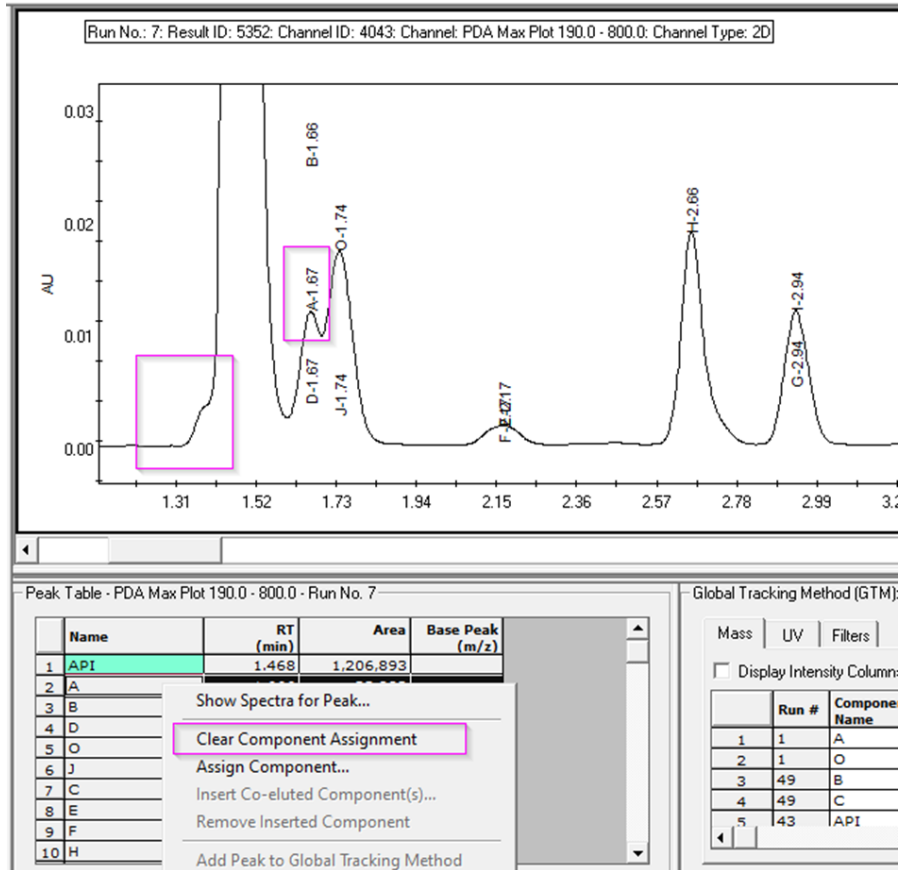
## Peak Tracking – Recommended Workflow after Data Import

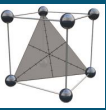
### Example for misassignment and ignored coelution

In the example chromatogram we can see a shoulder in the fronting of the API peak, clearly an ignored coelution.

In addition you can see component A has been assigned to the peak eluting directly after the API. From other runs we know, that this component in most case elutes directly before the API.

Select *Clear Component Assignment* for Component A in the peak table. As a result the entry in row 2 will get unassigned.





## Peak Tracking – Recommended Workflow after Data Import

### Example for misassignment and ignored coelution

- Now select the row for API and *Insert- Co-eluted component(s) ...*

Peak Table - PDA Max Plot 190.0 - 800.0 - Run No. 7

	Name	RT (min)	Area	Base Peak (m/z)
1	API	1.468	1,206,893	
2		1.666	33,882	
3	B	1.664		
4	D	1.666		
5	O	1.741	70,882	
6	J	1.741		
7	C	1.741		

Context menu for Row 1:

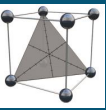
- Show Spectra for Peak...
- Clear Component Assignment
- Assign Component...
- Insert Co-eluted Component(s)...**

- A list of not yet assigned component names is given. Press OK, to select component A

Insert Co-eluted Component - Peak Table Row 1

Include	
<input checked="" type="checkbox"/>	A

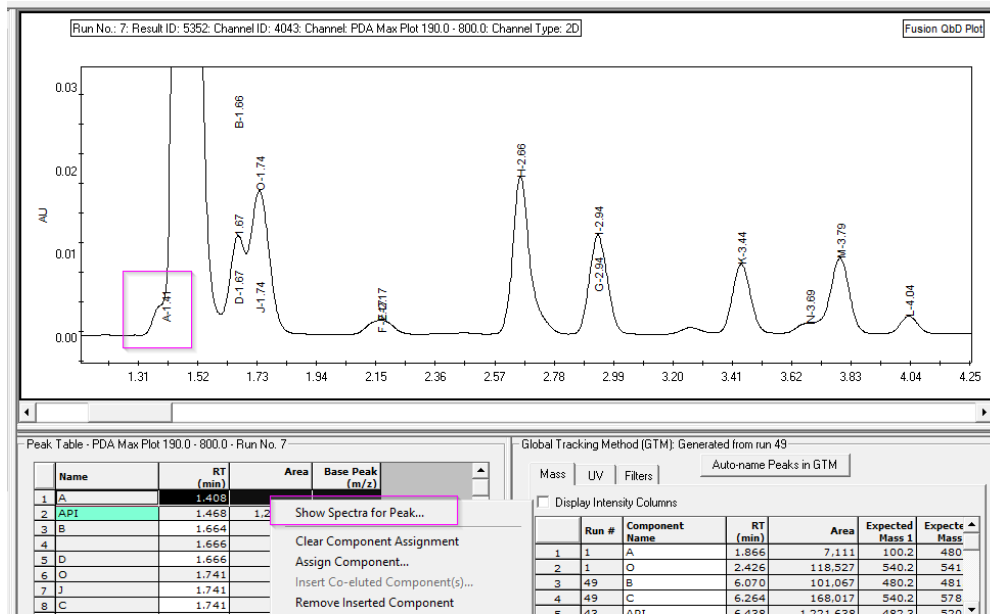
OK Cancel



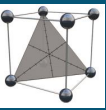
## Peak Tracking – Recommended Workflow after Data Import

### Example for misassignment and ignored coelution

- Component A is now assigned as a co-elution to the API peak.



- To confirm your decision you can now launch the Show Spectra for Peak ... command and check the spectral data for this component against the data in the GTM.



## Peak Tracking – Recommended Workflow after Data Import

### Example for Unnamed Peak assignment:

- The row for the peak in line 4 has no peak name anymore. We now still have to assign a component name. In this case component D has been identified as co-elution. We decide to make a proper assignment for component D.

Peak Table - PDA Max Plot 190.0 - 800.0 - Run No. 7

	Name	RT (min)	Area	Base Peak (m/z)
1	A	1.408		
2	API	1.468	1,206,893	
3	B	1.664		
4		1.666	33,882	
5	D	1.666		
6	O	1.741	70,882	
7	J	1.741		
8	C	1.741		
9	E	2.170	10,919	

Global Tracking Method (GTM): Generat

Mass UV Filters

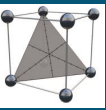
Display Intensity Columns

Component Name RT (min)

Context Menu:

- Show Spectra for Peak...
- Clear Component Assignment
- Assign Component...
- Insert Co-eluted Component(s)...
- Remove Inserted Component
- Add Peak to Global Tracking Method
- Ignore/Track Peak

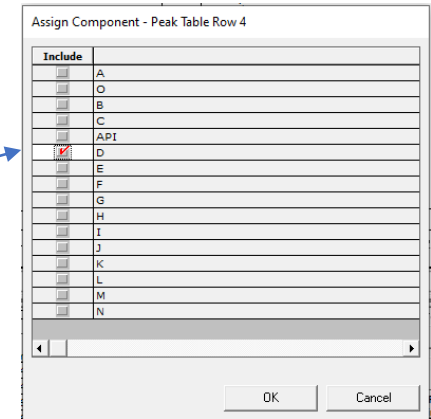
Synchronize Zoom and Formatting



## Peak Tracking – Recommended Workflow after Data Import

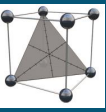
### Example for Unnamed Peak assignment:

- Launch the Assign Component ... command for line 4
- A list of all components is now given. Here you can select any component. Using a component name, that has already used for an assignment would perform a direct re-assignment. In our case we select component D.
- As a result component D is now directly assigned to the peak in line 4 and line 5 has been removed from the list.



Peak Table - PDA Max Plot 190.0 - 800.0 - Run No. 7

	Name	RT (min)	Area	Base Pe: (m/)
1	A	1.408		
2	API	1.468	1,206,893	
3	B	1.664		
4	D	1.666	33,882	
5	O	1.741	70,882	
6	J	1.741		
7	C	1.741		
8	E	2.170	10,919	
9	F	2.170		



## Basic Principles of the Peak Tracking Algorithm

The data from the **UV channel will be the base** for the peak tracking and subsequent data modelling and chromatogram predictions.

The **TIC channel is an auxiliary channel**. It is critical that peak integration and retention times are aligned with the UV channel. The retention times will be aligned by the retention time offset defined in the UV processing method.

The values for **Leading** and **Fronting** are applied to the peak integration in the UV channel. Values up to 50% can be set. The values indicate the position where the respective leading and trailing spectrum is extracted. The value represents the % distance between baseline start/end point and peak apex.

Spectral data will be imported from the 3D channels selected during data import. If PDA data are imported, the UV spectrum will be used for peaks that do not ionize. For peaks that show no absorbance in the UV channel, the TIC peak is used and will be simulated in a composite prediction chromatogram.

The GTM contains a **spectral fingerprint for each component**. For missing MS data, UV data will be used, listed on the UV tab. This can either be spectral data, if PDA data have been imported or traditional tracking data, if spectral data is missing at all.

During the tracking the PeakTracker will try to match all integrated peaks against the reference data given in the GTM. **Note, this includes any coelutions and the software will try to suggest an assignment for each compound defined in the GTM**



# Contact us for more information



Fusion QbD® is a mature LC method development software especially designed for AQbD approaches in the pharmaceutical industry.

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