DIA

Detailed description for Thermo Instruments (Q Exactive family):

Software requirements:

Foundation: 3.0 or newer
Xcalibur: 3.0 or newer
Q Exactive Orbitrap MS 2.3 SP1 or newer

Exemplary setup of a method in Xcalibur with 7 DIA segments:

1. Start a new method, enter your preferred LC settings for a i.e. 2h gradient, switch to the "Q Exactive Orbitrap MS" tab on the right (Figure 2).
2. Set the User Role to "Advanced" under "Global Settings".
3. Set the lock mass to best, the chrom. peak width to 30s and the method duration to the gradient length.
4. Generate one full MS –SIM scan group from the Experiments tab and extend its duration for the whole acquisition (130 min). Set its parameters (AGC, max. IT, …) as in Figure 2.
5. To generate the DIA windows for a simple method, we use a fixed DIA window width of 60 Th with 1 Th overlap and generate 7 in sequence starting from 500.

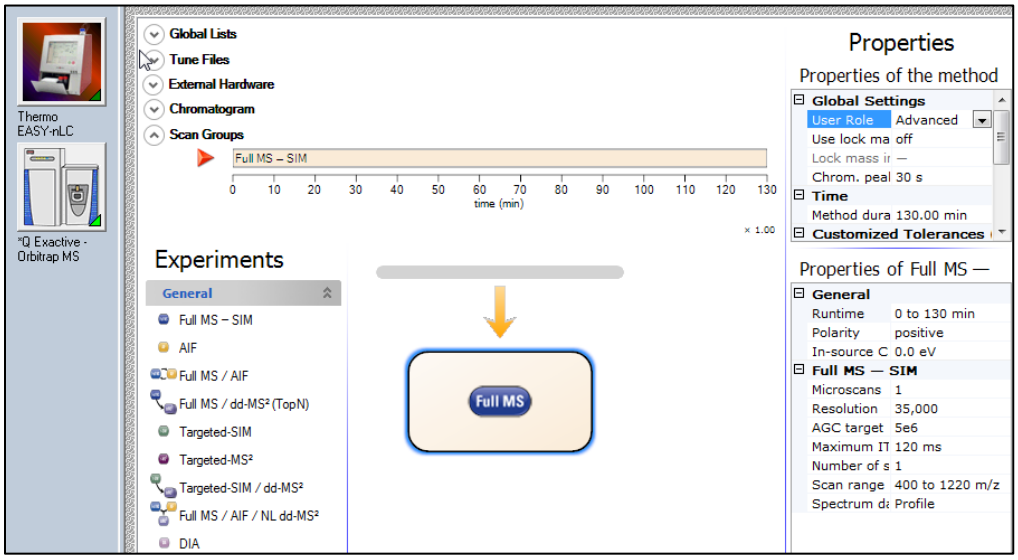

Figure 1: New DIA method

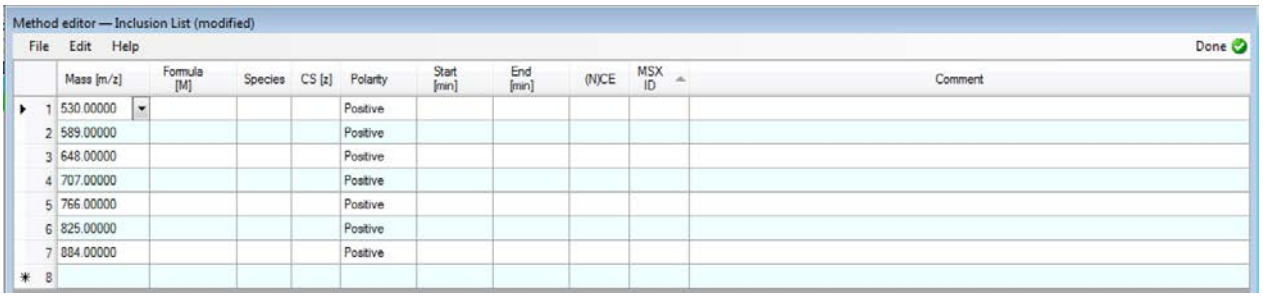

Figure 2: Global Inclusion List for a DIA method

Table 1: DIA windows with 1 Th overlap

DIA window	Start m/z	End m/z	Center	Width
1	500	560	530	60
2	559	619	589	60
3	618	678	648	60
4	677	737	707	60
5	736	796	766	60
6	795	855	825	60
7	854	914	884	60

6. Copy the window centers from Table 1 in “Global Lists” -> Inclusion list (Figure 2).
7. Insert one DIA scan group via the “Experiments” tab on the left and set the parameters according to the settings in Figure 4.
8. Copy the DIA scan group six times using drag and drop, to end up with a total of 7 DIA scan groups (Figure 3).
9. Change the “Isolation width” settings of the DIA scan groups as desired and keep them in sequential order according to the calculated widths of Table 1.
10. Save the method.

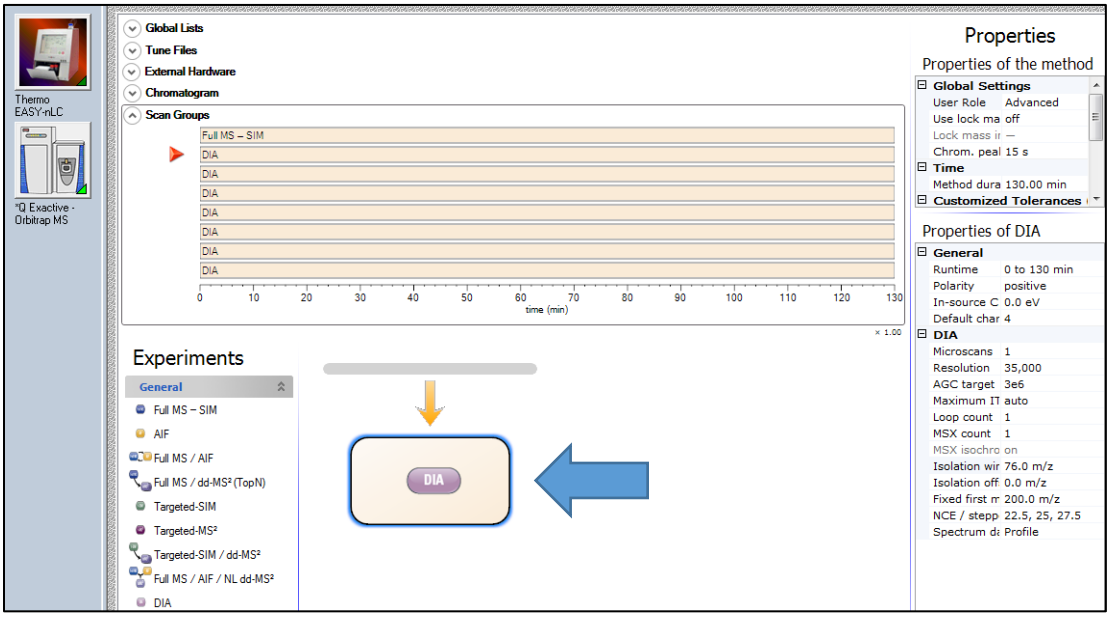

Figure 3: final DIA method. Use the mouse to drag the DIA scan group (blue arrow) to the orange DIA bar with the red arrow to generate additional DIA scan groups.