
Analyst MD Software

Information Dependent Acquisition Tutorial



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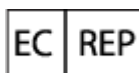
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Information Dependent Acquisition Tutorial

1

About IDA Methods

An IDA method automatically runs experiments based on results obtained from previous experiments in the same period. Use the IDA criteria to optimize data acquisition settings while acquiring data, which reduces the sample acquisition time in a single injection. IDA conserves both the amount of sample required and valuable working time.

Create an IDA method with up to two survey scans and eight dependent scans in a single period. A survey scan is used in IDA to trigger additional experiments. Any one of the following scans can be used as a survey scan:

- Q3 MS
- Enhanced MS (EMS)
- MRM
- Neutral Loss (NL)
- Precursor Ion (Prec)
- Enhanced Product Ion (EPI) (second-level survey scan)
- *Scheduled* MRM algorithm

The following are dependent scans:

- EPI
- MS/MS/MS (MS3) (second-level dependent scan)

In an IDA experiment, the mass spectrometer actions are varied from scan to scan based on the data acquired in a previous scan. The software analyzes data as it is being acquired and then determines the masses on which to perform dependent scans. Set the criteria that will activate an IDA experiment and the method parameters to be used.

IDA modifies experiments and improves results based on the following user-defined criteria:

- Ion intensity and charge state
- Inclusion and exclusion lists
- Isotope pattern

- Dynamic exclusion

If the polarity is not switched during the cycle, then the number of dependent experiments must match the number of most intense ions on the IDA Criteria tab.

Related Documentation

- System user guide for the mass spectrometer
- *Advanced User Guide*
- *Scheduled MRM Algorithm Tutorial*
- Analyst MD software Help

Prerequisites

Prerequisites
Users should be able to: <ul style="list-style-type: none">• Create an acquisition method• Submit a batch

Create IDA Methods

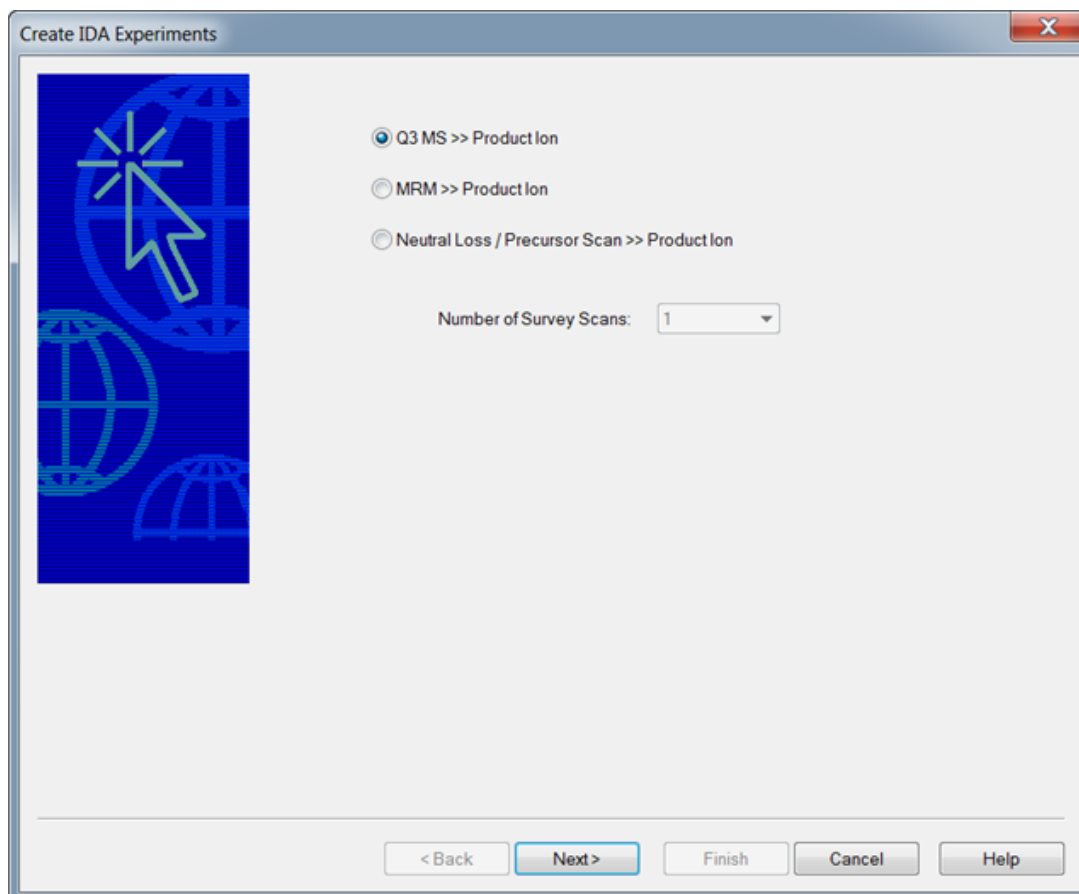
2

Use the IDA Method wizard to create linear ion trap (LIT) and triple quadrupole IDA acquisition methods. Any combination of two survey scans can be used. If EPI is selected as the survey scan, then the IDA–Second Level Criteria tab is available. If these survey experiments are used, then an additional IDA criteria level cannot be set.

Create an IDA Method using the IDA Method Wizard with a Triple Quadrupole System

1. On the Navigation bar, under **Acquire**, double-click **IDA Method Wizard**.

Figure 2-1 Create IDA Experiments Page



Create IDA Methods

2. Select the **Neutral Loss / Precursor Scan >> Product Ion** scan type.
3. In the **Number of Survey Scans** list, select the number of scans to be performed and then click **Next**.
The Survey Scan page is shown. The appearance of this page is dependent on the scan selected.
4. Set the parameters and then click **Next**.
The Dependent Scan – Product Ion Scan page is shown.
5. Select the number of peaks to monitor, set the scan parameters, and then click **Next**.
The Dependent Scan – IDA Criteria page is shown.

Note: Make sure that the IDA range is the same as, or within the range of, the survey scan.

6. To specify the ion mass range, type the lower and upper limits for the ion mass range. A dependent scan will be run for masses in this range.
7. In the **Which exceed** field, type the minimum number of counts per second required to trigger a dependent scan.
8. In the **Exclude Former Target Ions** group, do one of the following:
 - To ignore all former target ions occurring in the IDA experiment, select **Always**.
 - To ignore all former target ions after a specified number of occurrences, select **Always**, select **After**, and then type the number of occurrences.
 - To ignore former target ions for a specified period of time, select **For** and then type the number of seconds for which the ions will be ignored.
 - To ignore former target ions for a period of time after a number of occurrences, select **For** and then type the number of seconds for which the ions will be ignored. Select **After** and then type the number of occurrences.

Tip! To recognize all former target ions in the IDA experiment, complete the wizard, and in the IDA - First Level Criteria tab in the acquisition method editor, under **Exclude former target ions**, click **Never**.

9. To exclude former target ions after a specified number of occurrences, in the **Exclude Former Target Ions** group, select **After X occurrences** and then type the number of times that a former target ion can be seen before it is excluded from future scans.
10. Click **Finish**.
The IDA experiment is automatically shown in the Acquisition Method Editor. LC methods and other IDA criteria, for example inclusion and exclusion lists, can now be edited.
11. Review the MS method and edit parameters and other IDA criteria, if required.

12. Add the devices if they are not already included and then update all of the the relevant parameters.

Tip! All of the ion source gas parameters can be updated. In the MS tab, click **Edit Parameters**. On the Source/Gas tab, type the appropriate values and then select the **Source/Gas** check box at the bottom to apply the parameters to all the experiments.

13. Save the acquisition method file.

Create an IDA Method using the IDA Method Wizard with an LIT System

1. On the Navigation bar, under **Acquire**, double-click **IDA Method Wizard**. The Create IDA Experiments page is shown.
2. In the **Type of IDA Experiment** group, select the survey scan type and then click **Next**.
3. If the **Neutral Loss / Precursor Scan >> Enhanced Product** scan type is selected, then in the **Number of Survey Scans** list, select the number of scans to be performed.
4. To confirm that the IDA experiment has acquired data that matches the user-specified charge state or isotope pattern prior to performing the dependent scan, select the **Use Enhanced Resolution Scan to confirm Charge State and Isotope Pattern** check box.
5. To add an MS3 scan to the experiment, select the **Enable MS3 Experiment generation** check box.
6. To have the software dynamically calculate the appropriate fill time for the LIT, make sure that the **Dynamic Fill Time** check box is selected.
7. Click **Next**. The Survey Scan page opens. The appearance of this dialog varies depending on the survey scan selected.
8. Edit the parameters based on the scan type selected and then click **Next**. The Dependent Scan – Enhanced Product Ion (EPI) page opens.
9. Select the number of peaks to monitor, set the scan parameters, and then click **Next**. The Dependent Scan – IDA Criteria page is shown.
10. To specify the ion mass range, type the lower and upper limits for the ion mass range. A dependent scan will be run for masses in this range.
11. In the **Which exceed** field, type the minimum number of counts per second required to trigger a dependent scan.
12. To specify the charge state range for the IDA Experiment, select the **With charge state X to Y charges** check box and then click the charge states.

Create IDA Methods

Tip! The charge state is dependent on the proper determination of the isotope ratio. Therefore, an ER scan should be used to confirm the charge state.

13. To include peaks with unknown charge states, select the **Include unknowns** check box.
14. In the **Exclude Former Target Ions** group, do one of the following:
 - To ignore all former target ions occurring in the IDA experiment, select **Always**.
 - To recognize all former target ions occurring in the IDA experiment, select **Never**.
 - To ignore former target ions for a specified period of time, select **For** and then type the number of seconds for which the ions will be ignored.
 - To ignore former target ions for a period of time after a number of occurrences, select **For** and then type the number of seconds for which the ions will be ignored. Select **After** and then type the number of occurrences.
 - To ignore all former target ions after a specified number of occurrences, select **Always**, select **After**, and then type the number of occurrences.
15. To exclude former target ions after a specified number of occurrences, in the **Exclude Former Target Ions** group, select **After X occurrences** and then type the number of times that a former target ion can be seen before it is excluded from future scans.
16. Click **Finish**.
The IDA experiment is automatically shown in the Acquisition Method Editor. LC methods and other IDA criteria, for example inclusion and exclusion lists, can now be edited.
17. Review the MS method and edit parameters and other IDA criteria, if required.
18. Add the devices if they are not already included and then update all of the the relevant parameters.

Tip! All of the ion source gas parameters can be updated. In the MS tab, click **Edit Parameters**. On the Source/Gas tab, type the appropriate values and then select the **Source/Gas** check box at the bottom to apply the parameters to all the experiments.

19. Save the acquisition method file.

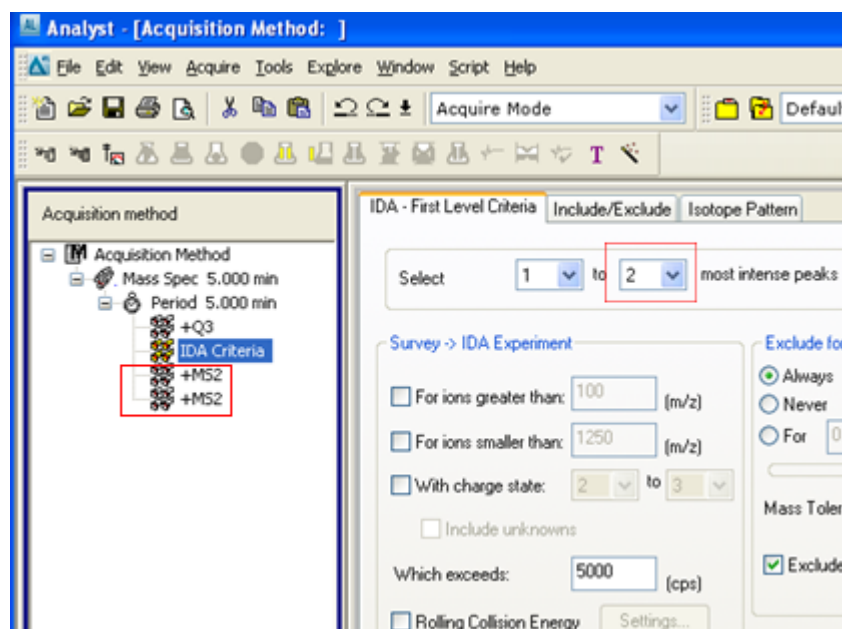
Create an IDA Method using the Acquisition Method Editor

Note: Experiments must be entered in the order that they are to be looped. Experiments cannot be inserted between existing experiments.

Tip! To reduce the need for polarity switching and minimize cycle time, group all positive dependent experiments together and all negative dependent experiments together within a period.

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.
2. Create a new acquisition method with one or two survey scans.
3. Select the survey scan and then select the appropriate parameters.
4. To use an Enhanced Resolution scan to confirm charge state or isotope pattern selection on a QTRAP system, do the following:
 - On the **Period** icon, right-click and then click **Add experiment**.
 - On the MS tab, in the **Scan type** list, click **Enhanced Resolution (ER)**.
 - Type the appropriate details for the Enhanced Resolution scan.
5. On the **Period** icon, right-click and then click **Add IDA Criteria Level**.
6. In the **Select X to Y most intense peaks** fields, set the range of the most intense ion peaks to be monitored by the IDA experiment to monitor. For single-survey IDA, the total number of peaks monitored must be equal to the number of dependent experiments specified. For dual-survey IDA with polarity switch in the survey scans, the number of dependent experiments must be double the total number of peaks to be monitored, with half of them in one polarity, and the other half in the opposite polarity.

Figure 2-2 Example of an IDA Method with Two Dependent Scan Types and Two Most Intense Ions



7. If an Enhanced Resolution (ER) scan is used, then add a placeholder for each peak monitored.
For ER placeholders, type 1 in the center (amu 0) window. If 4 peaks are monitored, then there will be 4 rows with the numbers 1, 2, 3, and 4 in the center column. For more information, refer to the section: [About Placeholders](#).
 8. If an ER scan is used to confirm charge state, isotope pattern selection, or regain mass accuracy, then select the **Use Enhanced Resolution Scan to confirm Charge State OR Isotope Pattern Selection** check box on the IDA - First Level Criteria tab.
 9. Set the rest of the parameters on the IDA - First Level Criteria tab to the required values.
 10. If required, on the Include/Exclude tab, modify the appropriate list to select the **Include List** or **Exclude List** check boxes and then specify the target ions or exclude ions.
 11. If required, on the Include/Exclude tab, select the **Match Isotopes** check box and then specify the isotopic distribution pattern to be matched.
 12. For the first dependent experiment to be added, one for each peak monitored by the first level IDA criteria, do the following:
 - a. On the **Period** icon, right-click and then click **Add experiment**.
An experiment is added below the IDA criteria.
 - b. Type the appropriate details for the dependent scan.
-
- Tip!** If more than one dependent scan will be used, right-click the EPI scan and then select **Copy this experiment** until the correct number of dependent scans are included. When copy is used instead of add experiment, all of the same settings are copied to the new dependent scans.
-
13. Open the method.
 14. Add the devices and update all the relevant parameters.
 15. Save the acquisition method file.

Create an IDA Method using a *Scheduled* MRM Algorithm Scan

For this example, create an IDA method that will look for the single most intense ion from the survey scan type. The acquisition method will contain one survey scan and one dependent scan.

To create a method that has multiple dependent scans, make sure that the number of intense peaks matches the number of dependent scans on the IDA Criteria tab (only applicable to

single-survey IDA). For example, if the method contains three dependent scans then select the 1 to 3 most intense peaks.

1. Create a *Scheduled* MRM experiment. Refer to the document: *Scheduled MRM Algorithm Tutorial*.

Note: When the Target Cycle Time option is selected for use in the *Scheduled* MRM experiment, then the target cycle time is only applicable to the *Scheduled* MRM experiment, and not to all of the experiments within the IDA acquisition method.

Tip! To add an ER scan type, add it before adding the IDA Criteria.

2. On the **Period** icon, right-click and then click **Add IDA Criteria Level**.
3. Specify the **IDA Criteria** parameters.
4. On the **Period** icon, right-click and then click **Add experiment**.
5. On the MS tab, in the **Scan type** list, select a dependent scan type. For this example, select **Product Ion (MS2)** or **Enhanced Product Ion (EPI)**.

Note: For all dependent scan types, the **Product Of** must be 30 Da.

6. Specify the experiment parameters.
7. Save the acquisition method in the project from which the acquisition will run.

Create an IDA Method using two *Scheduled* MRM Algorithm Scans

For this example, create an IDA method that looks for the two most intense ions from both survey scan experiments combined. The example acquisition method includes two survey scan experiments and four dependent scan experiments.

Tip! If the polarity is switched during the survey scan types, then refer to the section: [About Polarity Switching](#).

Note: For a dual-survey IDA acquisition method, two MRM experiments or two EMS (Enhanced MS) experiments can also be used for survey scans.

1. Create a *Scheduled* MRM experiment. Refer to step 1 to step 7 in *Create a Scheduled MRM Algorithm Acquisition Method* in the document: *Scheduled MRM Algorithm Tutorial*.
2. On the **Period** icon, right-click and then click **Add experiment**.
A second MRM scan is created. Set it to the opposite polarity.

Create IDA Methods

3. Select the **Enabled** check box in the **Scheduled MRM** group to create another *Scheduled MRM* experiment. Refer to the document: *Scheduled MRM Algorithm Tutorial*.
4. On the **Period** icon, right-click and then click **Add IDA Criteria Level**.
5. Specify the **IDA Criteria** parameters.
6. On the **Period** icon, right-click and then click **Add experiment**.
7. On the MS tab, in the **Scan type** list, select a dependent scan type. For this example, select **Product Ion (MS2)** or **Enhanced Product Ion (EPI)**.

Note: For all dependent scan types, the **Product Of** must be 30 Da.

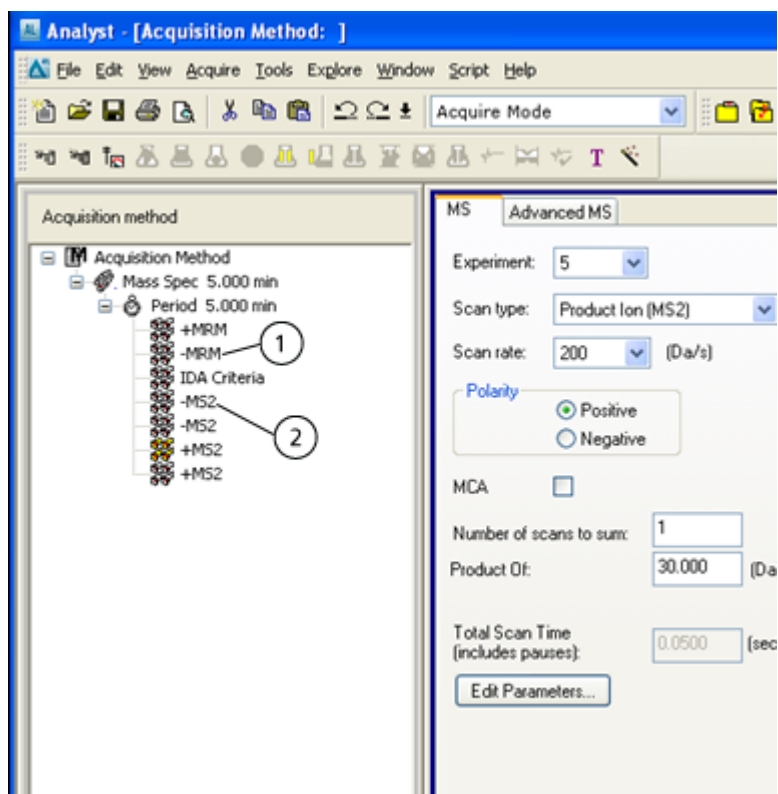
8. Specify the experiment parameters.
9. Right-click the **MS2** or **EPI** experiment and then click **Copy this experiment**.
10. Repeat step 9 twice. Make sure that the polarity is set as described in the section: [About Polarity Switching](#).
11. Save the acquisition method in the project from which the acquisition will run.

About Polarity Switching

If the survey scans are in different polarities, then make sure that the acquisition method is set up as follows:

1. Make sure that the polarity of the *first* dependent scan type is the same as the polarity of the *last* survey scan type.

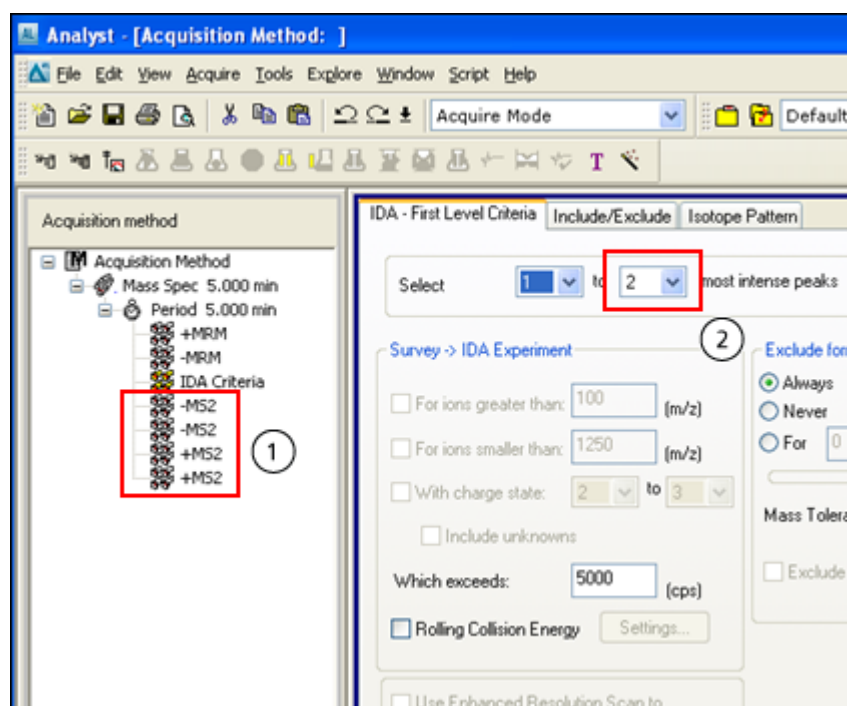
Figure 2-3 Example of Scan Types Set Up for Polarity Switching



Item	Description
1	Last survey scan
2	First dependent scan

2. Create an acquisition method in which the number of dependent scans is double the number of most intense ions. For example, to search for the two most intense ions, include four dependent scan types.

Figure 2-4 Example of Dependent Scan Types and Number of Most Intense Ions



Item	Description
1	Dependent scan types (four)
2	Most intense ions (two)

Note: The selected candidates are only triggered in the dependent experiments of the same polarity. The dependent scans where a candidate is not assigned are not run for this cycle. A maximum of half of the dependent experiments are triggered in any cycle.

- For each dependent scan experiment, make sure that if its polarity is the same as the previous experiment, then the settling time is set to zero.

About Placeholders

Placeholders are generic numbers used in scans that will be replaced by specific numbers. The software replaces the placeholder value with a specific value from the IDA selection process. Placeholders are critical for IDA methods to work properly.

When some experiments are first selected, placeholder values are automatically added.

- NL, Prec, and EPI = 30

- MS/MS/MS
 - First Precursor = 30
 - Second Precursor = 10

ER also uses placeholders.

- The placeholders start with 1 in the first row in an IDA method. Each consecutive row increases by 1 to a maximum of 8 rows.
- If the IDA Method Wizard is used, then it automatically adds the correct number of ER placeholders.

Impact of *Scheduled* MRM Pro Algorithm in IDA

If an Information Dependent Acquisition (IDA) survey scan is performed using the *Scheduled* MRM Pro algorithm, then a dependent scan in the IDA method is only triggered when the intensities of all of the MRM transitions in a group are above their trigger thresholds. This improves the cycle time by eliminating false triggers of dependent scans.

Create an IDA *Scheduled* MRM Pro Algorithm Acquisition Method

1. Create a *Scheduled* MRM Pro algorithm method. Refer to the document: *Scheduled MRM Tutorial*.
2. If required, add an experiment of ER scan type before adding an IDA criteria level in the period.
3. On the **Period** icon, right-click and then click **Add IDA Criteria Level**.
4. Specify the IDA Criteria parameters. Refer to the section: [Create IDA Methods](#).
5. On the **Period** icon, right-click and then click **Add experiment**.
6. On the MS tab, in the **Scan type** list, select a dependent scan type. For this example, select **Product Ion (MS2)** or **Enhanced Product Ion (EPI)**.

Note: For all dependent scan types, the **Product Of** must be 30 Da.

7. Specify the experiment parameters.
8. Save the acquisition method in the project from which the acquisition will run.

Note: During data acquisition using an IDA *Scheduled* MRM Pro algorithm method, the trigger threshold for each MRM transition in the method is used instead of the IDA threshold.

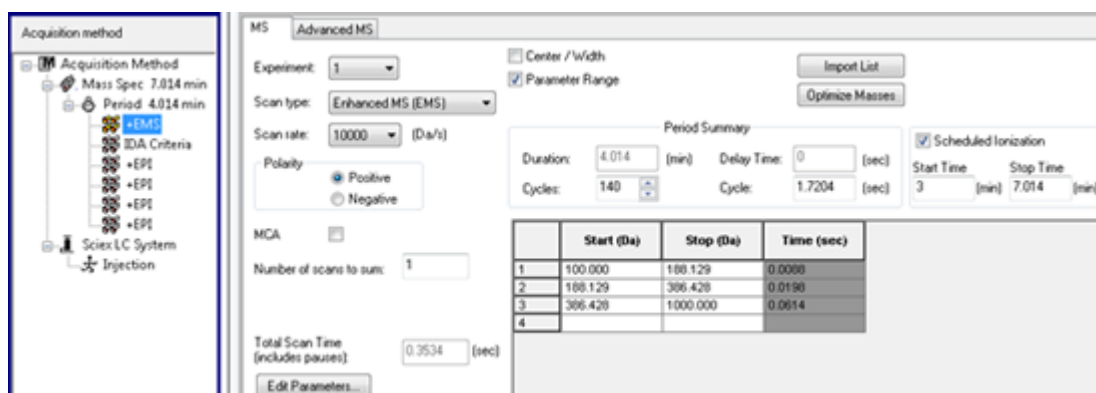
Create an IDA Method using Scheduled Ionization

1. Create an IDA method manually in the Method Editor or automatically using the **IDA Method Wizard**.
2. Select any of the experiments in the method, and then select the **Scheduled Ionization** check box.
3. In the **Scheduled Ionization** group, type appropriate **Start Time** and **Stop Time**. Make sure that the peaks of interest will elute between the **Start Time** and **Stop Time**. Also make sure that the **Synchronization Mode** and the LC portion of the acquisition method are set the same as when **Scheduled Ionization** is not used.

Note: **Scheduled ionization** is only available in single-period acquisition methods.

The following figure shows that the **LC Synchronization Mode** is used and the LC method is 10 minutes long. All of the peaks of interest elute after 3 minutes and before 7 minutes. An **IonSpray** voltage of 0 will be applied before the **Start Time** and after the **Stop Time**. The **IonSpray** voltage set in the method is only applied between the **Start Time** and **Stop Time**. The **Scheduled Ionization** feature can reduce the risk of instrument contamination and thus decrease the mass spectrometer down time. For more information about **Scheduled Ionization**, refer to the document: *Advanced User Guide*.

Figure 2-5 Scheduled Ionization



4. Save the acquisition method.

If data is being acquired using an IDA method, then the data does not open in the IDA viewer until the acquisition has finished. However, it does open in the Explore window during acquisition.

To make viewing IDA data easier, set the IDA Explorer as the default viewer. Use the IDA Explorer tab in the Appearance Options dialog to select the IDA Explorer that will be used to show IDA data. The user can also select the columns for the Mass-List List view pane. The IDA Explorer is preset to show IDA samples.

Set IDA Explorer Options

1. Click **Tools > Settings > Appearance Options**.
The Appearance Options dialog opens.
2. Open the IDA Explorer tab.
3. Select the **Use IDA Explorer to Display IDA Samples** check box.
4. In the **Column Options** section, select the following check boxes as required:

Table 3-1 Column Options

Field	Description
Intensity	Shows the intensity for a particular <i>m/z</i> ratio.
Molecular Weight (MW)	Shows the calculated molecular weight for a particular <i>m/z</i> ratio.
Scan	Shows the scan type used for a particular <i>m/z</i> ratio.
Collision Energy (CE)	Shows the collision energy for a particular <i>m/z</i> ratio.
Charge (Z)	Shows the charge for a particular ion.

5. For an LIT system, in the **LIT Column Options** section, select the following check boxes as required:

Table 3-2 LIT Column Options

Field	Description
Excitation Energy (AF2)	Shows the excitation energy for the second precursor ion in MS/MS/MS experiments.

View IDA Data

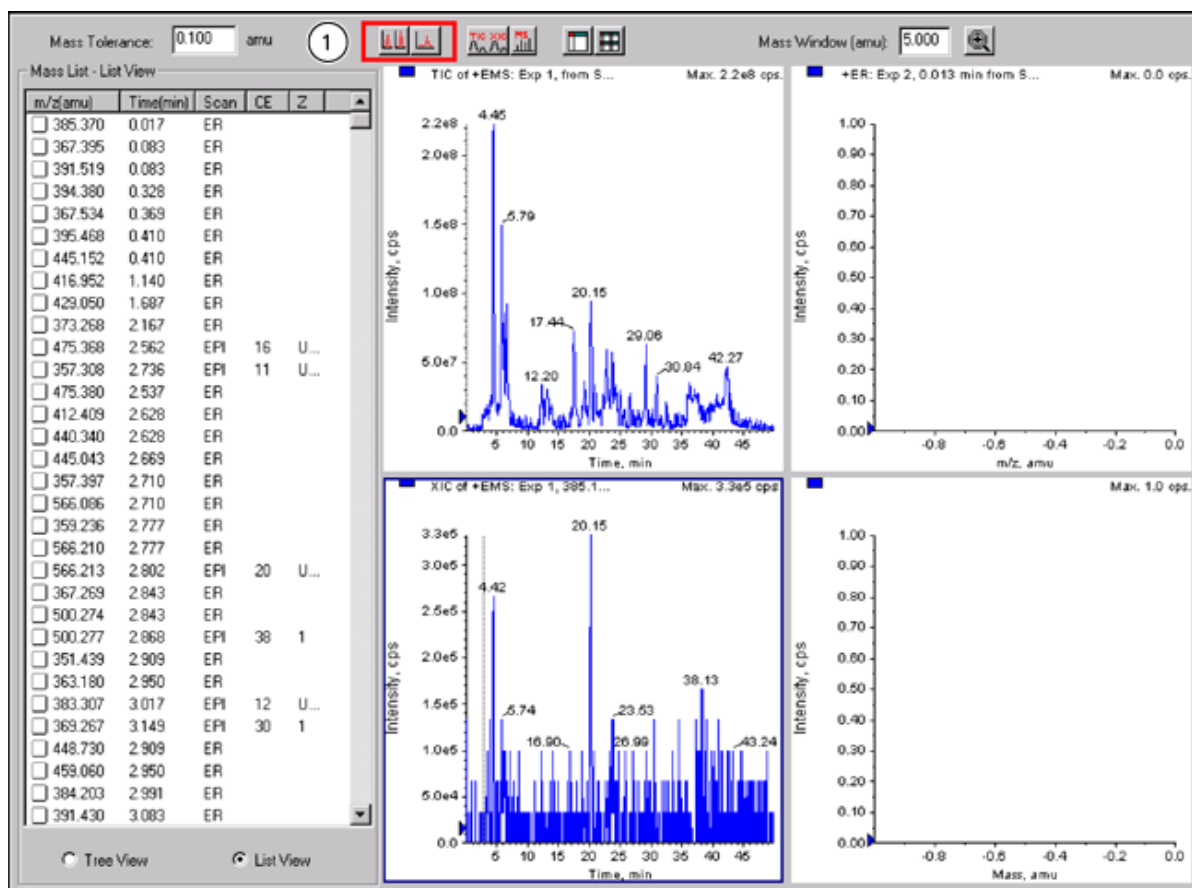
Table 3-2 LIT Column Options (continued)

Field	Description
MS3 2nd Precursor (2nd Pre)	Shows the MS/MS/MS second precursor ion.
DFT (Dynamic Fill Time)	Shows the time taken to fill the LIT.

View IDA Data

1. Make sure the Example project is selected in the Analyst MD software.
2. On the Navigation bar, under **Explore**, double-click **Open Data File**. The Select Sample dialog opens.
3. In the **Data Files** field, double-click **LIT** and then click **IDA BSA Digest.wiff**.
4. In the **Samples** list, click a sample, and then click **OK**.
The IDA Explorer opens. The left portion of the pane shows a list of masses sent to the dependent scan. To switch between views, select **Tree View** or **List View** at the bottom of the Mass List pane. The right portion of the pane contains graphical information about the IDA data.

Figure 3-1 Single Graph and Multiple Graph Display Buttons



5. Use the two buttons (item 1) above the graph to change between single graph and multiple graph. To view the active graph only, click the button that shows one graph. To return to multi-graph view, click the button that shows two graphs.

Tip! To access **Explore** mode functions that are not available in the IDA Viewer, click **Export Active Graph to Explorer** and **Export All Graphs to Explorer** in the pane above the graphs to open the current graphs in the Explore window.

View IDA Data if IDA Explorer is not Active

If the IDA Explorer is not active, then the IDA data opens in the Explore window. To view the IDA data, double-click the green arrow at the bottom of the X-axis.

View IDA Data

Figure 3-2 TIC Containing all Scans (IDA data arrow circled)

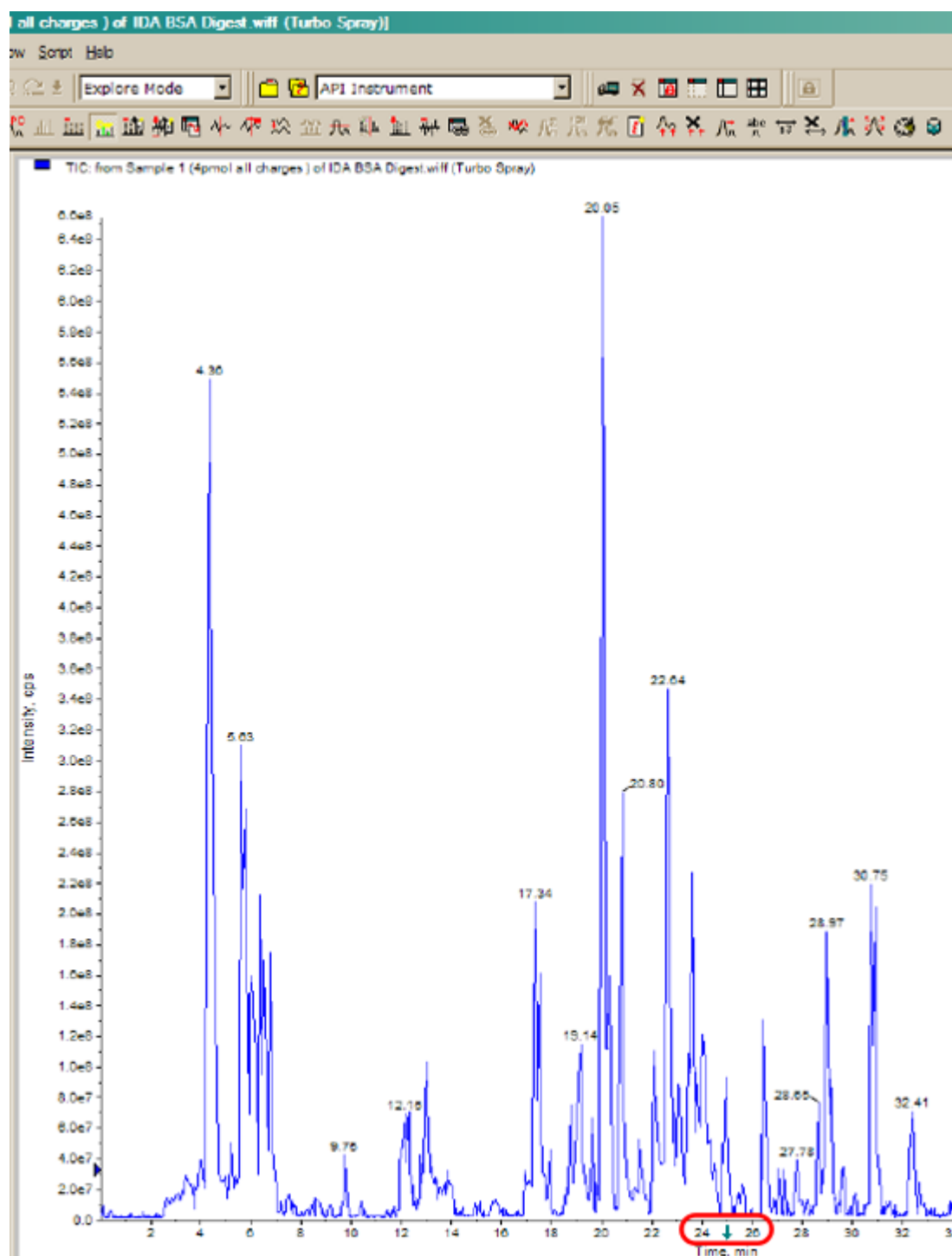
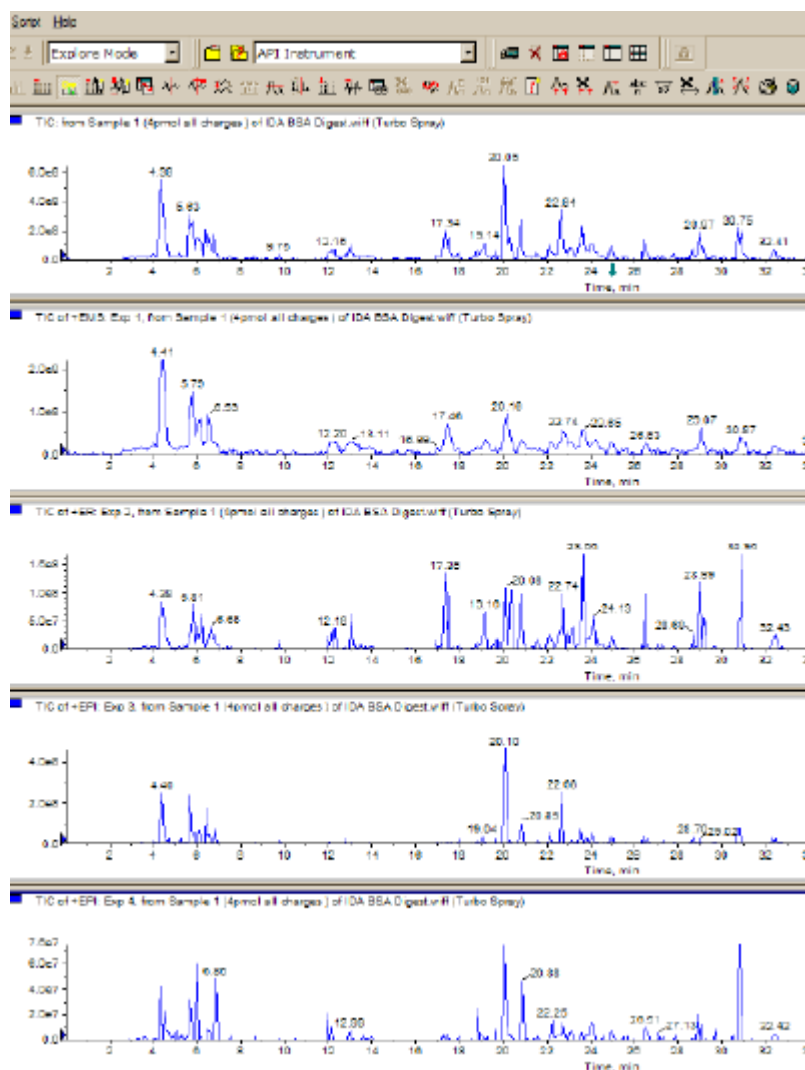


Figure 3-3 Individual and Additive TIC Scans



About the Dynamic Background Subtraction Algorithm

The Dynamic Background Subtraction (DBS) algorithm improves detection of precursor ions in an IDA experiment. When DBS is activated by selection of the **After Dynamic Background Subtraction of Survey scan** check box in the IDA – First Level Criteria tab, IDA uses a spectrum that has been background subtracted to select the candidate ion of interest for MS/MS analysis, instead of selecting the precursor from the survey spectrum directly. Because this process takes place during LC analysis, DBS enables detection of species as their signal

increases in intensity, thereby focusing on detection and analysis of the precursor ions on the rising portion of the LC peak, up to the top of the LC peaks (maximum intensity).

When DBS is used in an IDA method with the *Scheduled* MRM algorithm as survey scans, then the DBS option is not available in the *Scheduled* MRM experiments but it is used to trigger both the secondary transitions and dependent scans.

About Dynamic Fill Time

DFT (dynamic fill time) is a feature specifically designed to optimize the data obtained in every spectrum for the LIT functions. DFT automatically adjusts the time used to fill the LIT based on the ion flux coming from the source.

For more intense ions, the fill time is automatically reduced to make sure that the LIT is not overfilled with ions. For less intense ions, the fill time is automatically increased to make sure that good ion statistics are obtained in the spectrum. DFT is applicable for the following scan types:

- Enhanced MS (EMS)
- Enhanced Resolution (ER)
- Enhanced Product Ion (EPI)
- MS/MS/MS (MS³)

Users can adjust the DFT settings by selecting **Tools > Settings > Method Options** in the Analyst MD software.

Icons

Table 3-3 Acquisition Method Editor Icons









Icon	Name	Description
	Mass Spec	Click to show the MS tab in the Acquisition Method editor.
	Period	Right-click to add an experiment, add an IDA Criteria Level , or delete the period.
	Autosampler	Click to open the Autosampler Properties tab.
	Syringe Pump	Click to open the Syringe Pump Properties tab.

Table 3-3 Acquisition Method Editor Icons (continued)

Icon	Name	Description
	Column Oven	Click to open the Column Oven Properties tab.
	Valve	Click to open the Valve Properties tab.
	DAD	Click to open the DAD Method Editor.
	ADC	Click to open the ADC Properties tab.

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- [SCIEX Now Learning Hub](#)

SCIEX Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

- sciex.com/contact-us
- sciex.com/request-support

CyberSecurity

For the latest guidance on cybersecurity for SCIEX products, visit sciex.com/productsecurity.

Documentation

This version of the document supersedes all previous versions of this document.

To view this document electronically, Adobe Acrobat Reader is required. To download the latest version, go to <https://get.adobe.com/reader>.

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

To find hardware product documentation, refer to the *Customer Reference* DVD that comes with the system or component.

Note: To request a free, printed version of this document, contact sciex.com/contact-us.
