

# Analyst<sup>®</sup> 1.6 Software

*Peptide and Protein Quantitation Tutorial*



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# Peptide and Protein Quantitation Tutorial

Absolute quantification of peptides refers to the simultaneous detection and peak integration of MRM transitions corresponding to representative proteolytic peptides of unknown concentrations and synthetic analogues derivatized with stable isotopes introduced into the sample in known amounts for direct measurements.

Absolute quantification of peptides using stable-isotope coded amino acids (sometimes referred to as AQUA) is an effective application whereby the direct detection and quantification of both the native “light” peptide MRM transitions and the “heavy” synthetic internal standard MRM transitions are measured in the same experiment. Use the MultiQuant™ software 1.0 for data processing.

Topics in this section:

- [Related Documentation on page 3](#)
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## Related Documentation

- *Getting Started Guide* for the Analyst® software
- *Software Reference Guide* for the Analyst software
- NanoSpray® II or III ion source operator’s guide
- MRMPilot™ software manual

## Technical Support

AB SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the instrument or any technical issues that may arise. For more information, visit the Web site at [www.absciex.com](http://www.absciex.com).

## Prerequisites

You should be able to:

- Create a hardware profile.
- Create an acquisition method using the Analyst® software.
- Create an LC method.

- Submit a batch.

## Creating an MRM Method using the MIDAS™ Workflow for Peptide Quantitation

1. Make sure that you are in the correct project.
2. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.
3. In the **Acquisition method** pane, click **Mass Spec** and then, on the **MS** tab, select the following parameters.

**Table 1-1 Acquisition Method Parameters**

Parameter	Value
Scan type	MRM
Polarity	Positive
Duration	Chromatography run time.

4. On the **MS** tab, type the data in the following table into the mass ranges table. Right-click in the mass ranges table to add the compound-dependent parameters as shown in [Table 1-2](#) and [Figure 1-1](#).



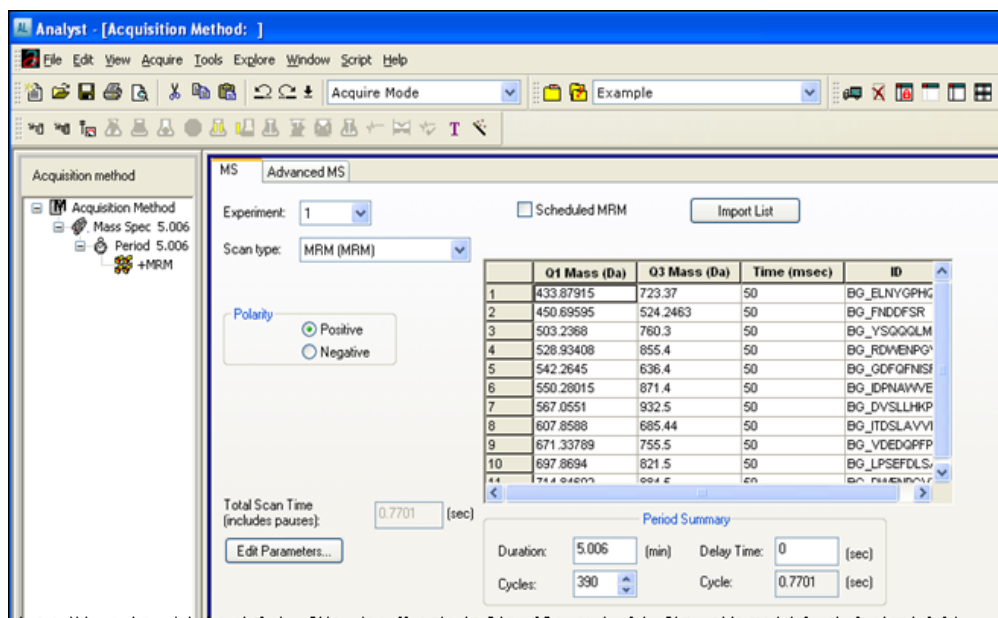
**Note:** You can also import data into the mass ranges table. If large numbers of MRM methods need to be created for proteins and peptides, then the MRMPilot™ software can be used. For more information, see documentation that comes with the MRMPilot software or see [Importing from Excel on page 10](#).

**Table 1-2 Mass Ranges and Compound Parameters**

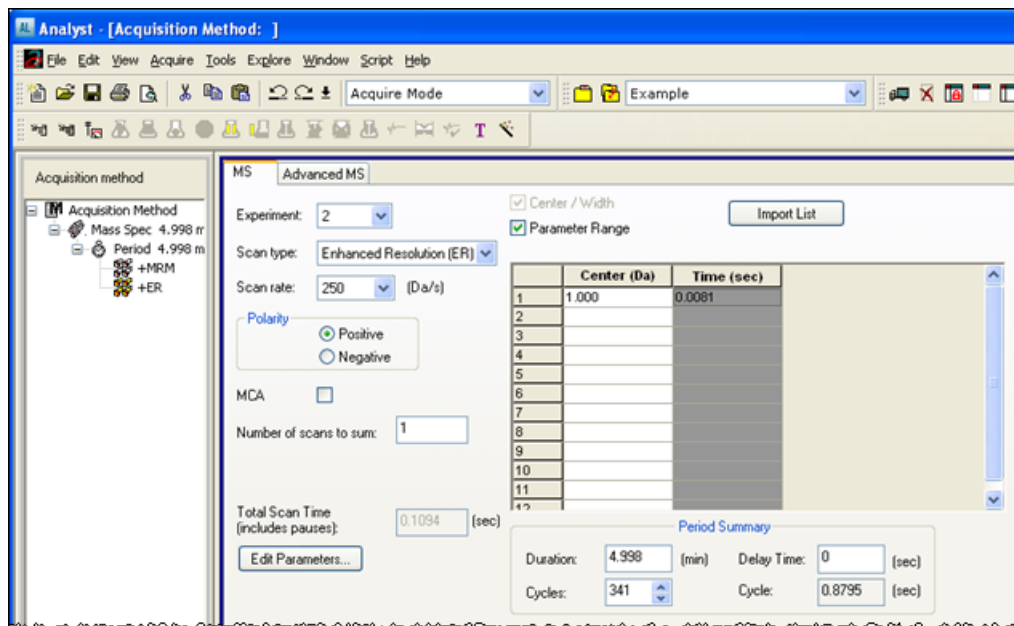
Q1	Q3	Time (msec)	ID	CE
433.87915	723.37	50	BG_ELNYPHQWR	30
450.69595	524.2463	50	BG_FNDDFSR	28
503.2368	760.3	50	BG_YSQQQLMETSHR	27
528.93408	855.4	50	BG_RDWENPGVTQLNR	25
542.2645	636.4	50	BG_GDFQFNISR	26
550.28015	871.4	50	BG_IDPNAWVER	27
567.0551	932.5	50	BG_DVSLHLKPTTQISDFHVATR	30
607.8588	685.44	50	BG_ITDSLAVVLQR	39
671.33789	755.5	50	BG_VDEDQPFPAVPK	33
697.8694	821.5	50	BG_LPSEFDLSAFLR	35
714.84692	884.5	50	BG_DWENPGVTQLNR	32

**Table 1-2 Mass Ranges and Compound Parameters (Continued)**

Q1	Q3	Time (msec)	ID	CE
729.36517	832.5	50	BG_APLDNDIGVSEATR	48
871.9516	915.5	50	BG_LSGQTIEVTSEYLFR	40
879.4339	664.3	50	BG_VNWLGLGPQENYPDR	40

**Figure 1-1 MRM scan type**

- To add another experiment to the IDA method, right-click the MRM row in the left pane and then click **Add experiment**.  
A new row is added to the method.
- Click the new row and then change the **Scan type** to **Enhanced Resolution**. In the field for masses on the right, type the value 1 in the first row, which indicates to the software that it is an IDA method and to use the most intense MRM transition to trigger acquisition of a trap MS/MS scan. If more than one precursor ion is to be selected in each cycle, add an extra row for each precursor ion to be selected in the Enhanced Resolution scan type and number the rows 1, 2, 3 and so forth. You can select up to eight precursor ions in an IDA cycle.
- Select a scan speed. A scan speed of 250 Da/s is typical for both the 4000 QTRAP<sup>®</sup> system and the AB SCIEX QTRAP 5500 system.



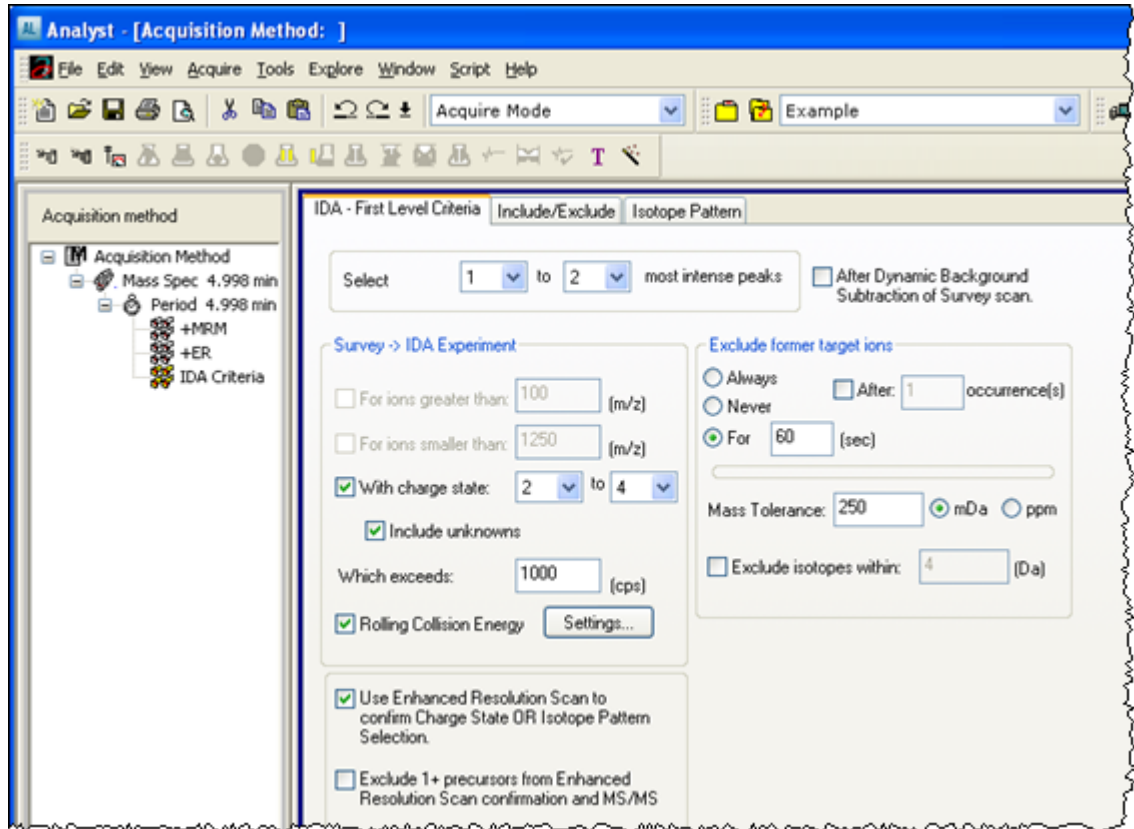
**Figure 1-2 ER scan type**

8. Click the **Advanced MS** tab and make sure that **Dynamic Fill Time** is selected.
9. Select **Q0 Trapping** in this scan so ions are trapped in Q0 for the first EPI scan. This will not affect the DFT scan for ER.



**Note:** If you are using the AB SCIEX QTRAP 5500 system to analyze higher abundant proteins, do not select the Q0 Trapping check box on the Advanced MS tab.

10. Right-click the **ER** scan type in the left pane and then select **Add IDA Criteria Level**. Typical parameters for IDA are shown in [Table 1-3](#).

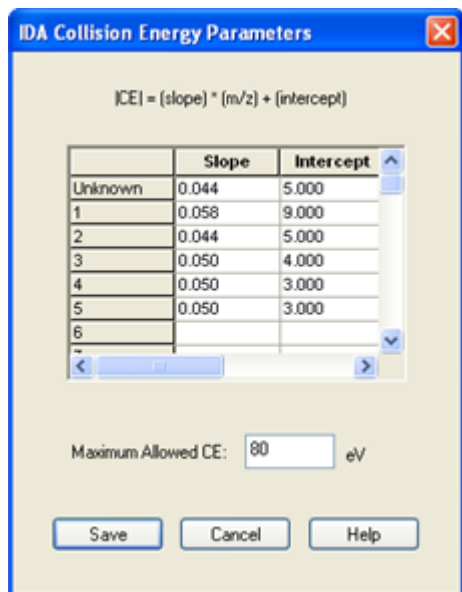


**Table 1-3 IDA Criteria Parameters**

Parameter	Value
Charge state _ to _ (for the predicted precursors)	2 to 4 for tryptic peptides
Which exceeds	(Intensity threshold) Determined by the estimated signal expected in cps (for example, 1000 cps)
Exclude former target ions	(Dynamic exclusion) Exclusion for 30 to 60 seconds is typical of the LC peak width and selecting after 1 occurrence to reflect exclusion after one MS/MS scan per cycle.
Use Enhanced Resolution scan to confirm Charge State	Selected
Use Rolling Collision Energy	Selected

- Click **Settings** beside **Rolling Collision Energy** and then type the settings shown in [Figure 1-3](#).

These values determine the collision energy used for MS/MS for each peptide precursor given its charge state.



**Figure 1-3 IDA CE Parameters**



**Note:** If there is no ER scan in the method, then the MS/MS acquisition is performed using the collision energy for an unknown charge state. This is typically set to the value of the 2+ charge state.

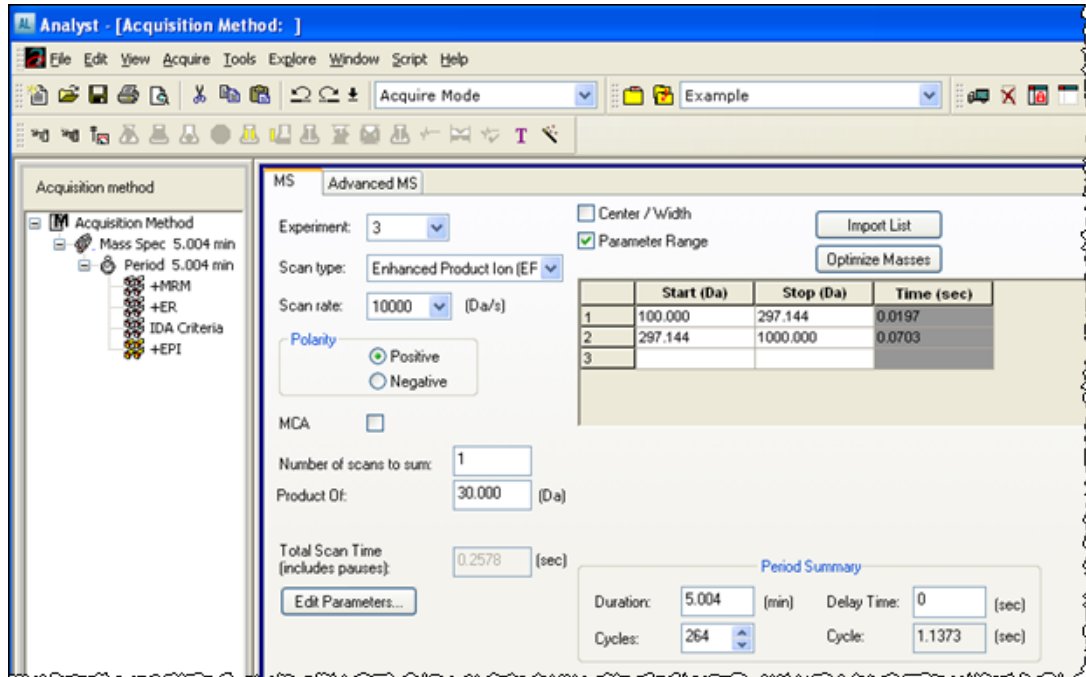
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12. Right-click **IDA Criteria** and then add another experiment. Change the **Scan type** to **Enhanced Product Ion**.
13. On the **MS** tab, type the parameters as shown in [Table 1-4](#).



**Note:** When you enter the minimum and maximum mass ranges, the software automatically optimizes the masses.

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**Table 1-4 MS Tab Parameters**

Parameter	4000 QTRAP system	AB SCIEX QTRAP 5500 system
Mass Range	100 to 1500 m/z	100 to 1000 m/z
Number of scans to sum	2	1
Scan Rate	4000 Da/s	10000 Da/s

14. Click the **Advanced MS** tab and then set the parameters as shown in [Table 1-5](#).



**Note:** If you are using the AB SCIEX QTRAP 5500 system to analyze higher abundant proteins, you should select the Dynamic fill time feature instead of Q0 Trapping on the Advanced MS tab.

**Table 1-5 Advanced MS Tab Parameters**

Parameter	4000 QTRAP system	AB SCIEX QTRAP 5500 system
Scan mode	Profile	Profile
Step size	0.12	0.12
Resolution Q1	Unit	Unit
Fixed LIT fill time	25 msec	5 msec
Q0 Trapping	Yes	Yes

15. Click **Add experiment** and then add up to eight EPI experiments per cycle. Use the parameters from [Table 1-4](#) and [Table 1-5](#). The recommended number of EPI scans

per cycle is one for the 4000 QTRAP system and three for the AB SCIEX QTRAP 5500 system.

16. Save the method.

## Importing from Excel

You can import a list of previously determined MRM transitions directly into the starter method. The exported spreadsheet must be saved as a tab delimited text file.

1. Create the MRM transition list in Excel and then save the list as a tab delimited text file. Close the txt file before importing.




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**Note:** When importing a text file, the column format of the .txt file must exactly match the column format in the text file.

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2. On the **MS** tab in the **MRM** scan type, click **Import List**.
3. Navigate to the .txt file and then click **Open** to import the MRM transitions.

## Creating an MRM Method for Peptide Quantitation




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**Note:** If you are creating MRM assays for isotope-labeled peptides, then use the MRMPilot™ software.

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1. Make sure that you are in the correct project.
2. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.
3. In the **Acquisition method** pane, click **Mass Spec**.
4. On the **MS** tab, type the parameters from the following table.

**Table 1-6 Acquisition Method Parameters**

Parameter	Value
Scan type	MRM
Polarity	Positive
Duration	Chromatography run time

5. In the mass ranges table, type the MRM Q1/Q3 transitions, the dwell time, ID, and CE value corresponding to the peptides as shown in [Table 1-7](#):

**Table 1-7 Mass Table Values**

Q1	Q3	Time (msec)	ID	CE
503.2368	760.3	50	BG_YSQQQLMETSHR	27
542.2645	636.4	50	BG_GDFQFNISR	26

**Table 1-7 Mass Table Values (Continued)**

Q1	Q3	Time (msec)	ID	CE
671.33789	755.5	50	BG_VDEDQPFPAVPK	33
714.84692	884.5	50	BG_DWENPGVTQLNR	32
729.36517	832.5	50	BG_APLDNDIGVSEATR	48

6. Save the method.

## Creating a *Scheduled* MRM™ Algorithm Method

1. Make sure that you are in the correct project.
2. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.
3. In the **Acquisition method** pane, click **Mass Spec** and then, on the **MS** tab, select the following:

**Table 1-8 Acquisition Method Parameters**

Parameter	Value
Scan type	MRM
Polarity	Positive
Duration	Chromatography run time

4. Select **Scheduled MRM** to enable the *Scheduled* MRM algorithm.
5. Right-click in the mass range table and then click **Collision Energy CE**.

This adds an additional column to that table where you can type a collision energy for each row.

In the MRM transition table, the time column represents the expected retention time of the corresponding peptide in minutes.

6. On the **MS** tab, type the data from the following table into the mass ranges table. Right-click in the mass ranges table to add the compound-dependent parameters.

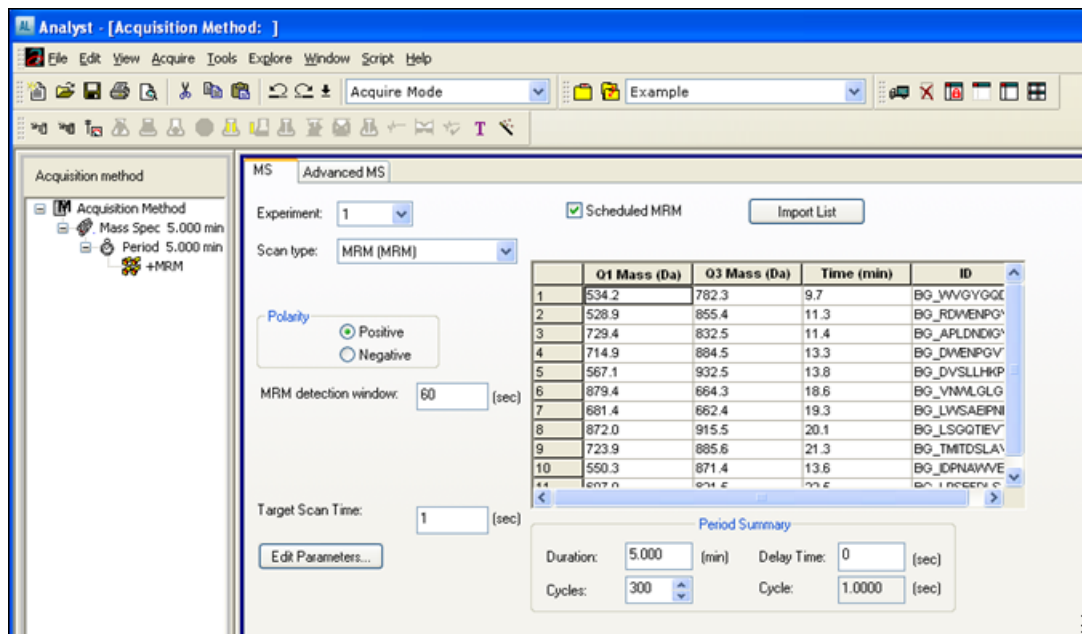


**Note:** You can also import data into the mass ranges table. For more information, see [Importing from Excel on page 10](#).

**Table 1-9 Mass Ranges and Compound Parameters**

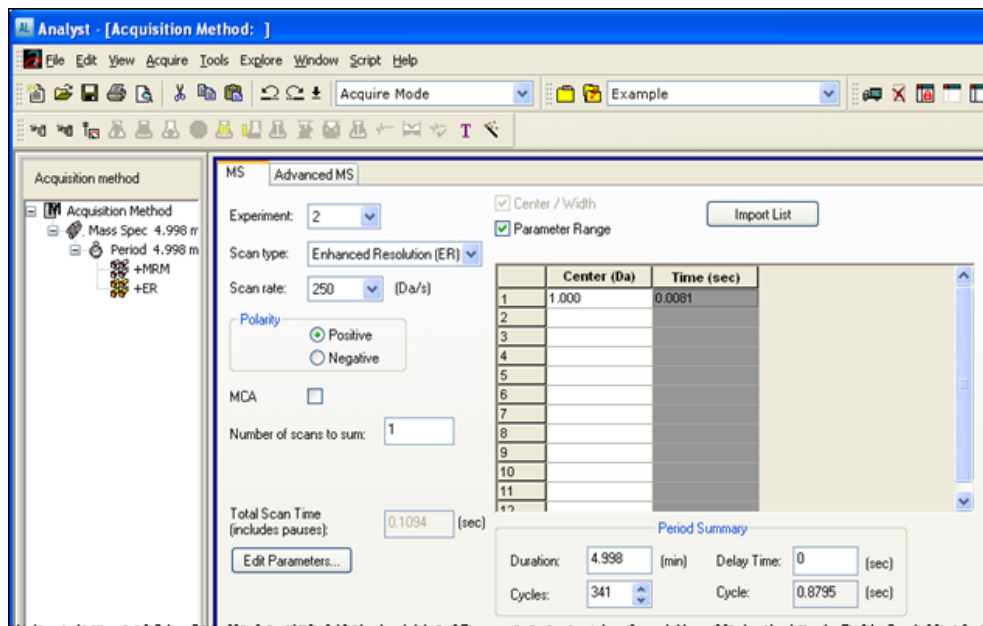
Q1	Q3	Retention Time (min) *	ID	CE
534.2	782.3	9.7	BG_WVGYGQDSR	23
528.9	855.4	11.3	BG_RDWENPGVTQLNR	25
729.4	832.5	11.4	BG_APLDNDIGVSEATR	48
714.9	884.5	13.3	BG_DWENPGVTQLNR	32
567.1	932.5	13.8	BG_DVSL LHKPTTQISDFHVATR	30
879.4	664.3	18.6	BG_VNWLGLGPQENYPDR	40
681.4	662.4	19.3	BG_LWSAEIPNLYR	30
872.0	915.5	20.1	BG_LSGQTIEVTSEYLFR	40
723.9	885.6	21.3	BG_TMITDSLAVVLQR	37
550.3	871.4	13.6	BG_IDPNAWVER	27
697.9	821.5	22.5	BG_LPSEFDLSAFLR	35
542.3	636.4	14.7	BG_GDFQFNISR	26
671.4	755.5	13.0	BG_VDEDQPFPVAVPK	33
495.4	690.3	14.0	BG_WLPAMSER	24
503.4	760.3	8.9	BG_YSQQQLMETSHR	27
<p><b>* These are typical retention times observed for a 20 minute chromatography gradient from 5 to 40% B [solvent B = 98% ACN + 0.1% formic acid. Retention times must be determined experimentally and then added to the table for the chromatography being used.</b></p>				

7. Type the MRM detection window in seconds applied to each MRM transition. For example, an MRM transition corresponding to a peptide expected to elute at 21 minutes with an MRM detection window set at 60 seconds means that the acquisition of that MRM transition will take place between 20.5 and 21.5 minutes. This parameter should be set as narrow as possible according to the reproducibility of the chromatography.



**Figure 1-4 Scheduled MRM algorithm scan type**

8. The Target Scan Time value represents the maximum cycle time to scan all MRMs in a given detection window. This should be the minimum time necessary to scan each MRM in a given detection window to achieve 10 to 15 scanned points across the eluting peak.
9. To add another experiment to the IDA method, right-click the MRM row in the left pane and then click **Add experiment**.  
A new row is added to the method.
10. Click the new row and then change the **Scan type** to **Enhanced Resolution**. In the field for masses on the right, type the value 1 in the first row, which indicates to the software that it is an IDA method and to use the most intense MRM transition to trigger acquisition of a trap MS/MS scan. If more than one precursor ion is to be selected in each cycle, add an extra row for each precursor ion to be selected in the Enhanced Resolution scan type and number the rows 1, 2, 3 and so forth. You can select up to eight precursor ions in an IDA cycle.
11. Select a scan rate. A scan rate of 250 Da/s is typical for both the 4000 QTRAP<sup>®</sup> system and the AB SCIEX QTRAP 5500 system.



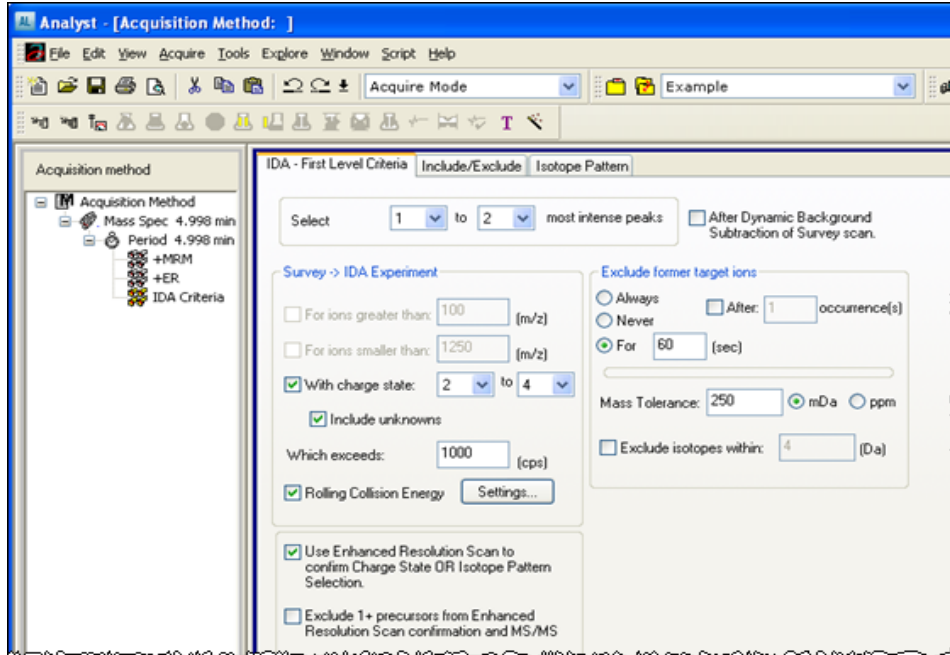
**Figure 1-5 Enhanced Resolution scan type**

12. Click the **Advanced MS** tab and make sure that **Dynamic Fill Time** is selected.
13. Select **Q0 Trapping** in this scan so ions are trapped in Q0 for the first EPI scan. This will not affect the DFT scan for ER.



**Note:** If you are using the AB SCIEX QTRAP 5500 system to analyze higher abundant proteins, do not select the Q0 Trapping check box on the Advanced MS tab.

14. Right-click the **ER** scan type in the left pane and then select **Add IDA Criteria Level**. Typical parameters for IDA are shown in [Table 1-10](#).



**Table 1-10 IDA Criteria Parameters**

Parameter	Value
Charge state _ to _ (for the predicted precursors)	2 to 4 for tryptic peptides
Which exceeds	(Intensity threshold) Determined by the estimated signal expected in cps (for example, 1000 cps)
Exclude former target ions	(Dynamic exclusion) Exclusion for 60 to 120 seconds is typical of the LC peak width and selecting after 1 occurrence to reflect exclusion after one MS/MS scan per cycle.
Use Enhanced Resolution scan to confirm Charge State	Selected
Use Rolling Collision Energy	Selected

- Click **Settings** beside **Rolling Collision Energy** and then type the settings shown in [Figure 1-6](#).

These values determine the collision energy used for MS/MS for each peptide precursor given its charge state.

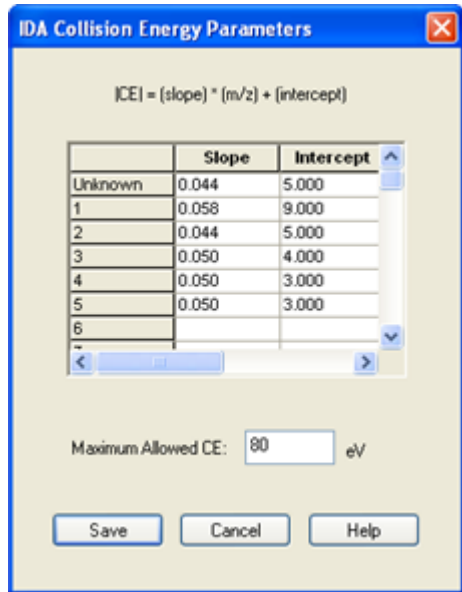
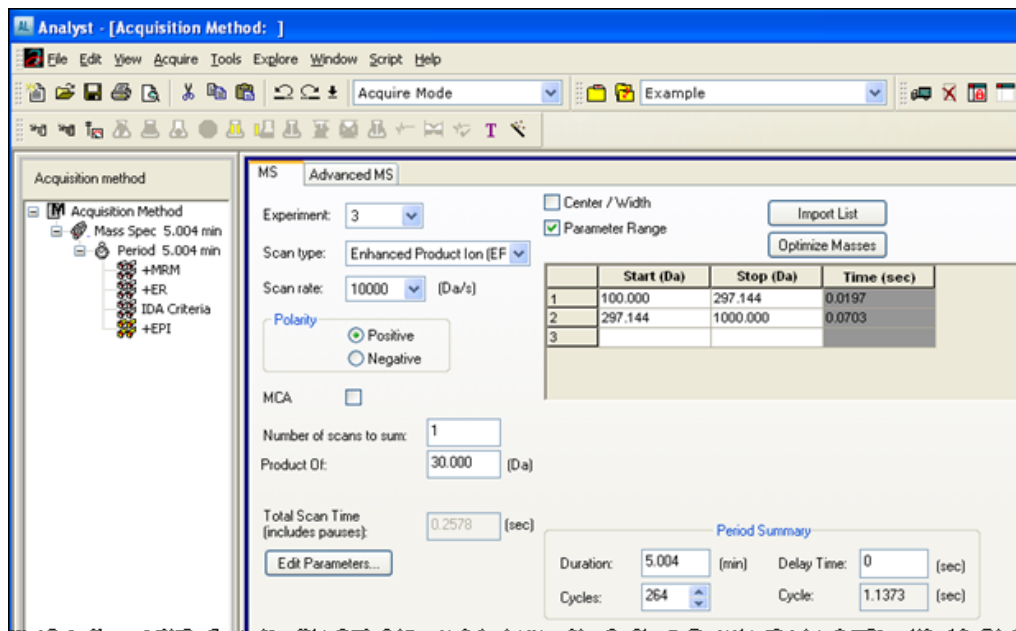


Figure 1-6 IDA CE Parameters



**Note:** If there is no ER scan in the method, the MS/MS acquisition is performed using the collision energy for an unknown charge state. This is typically set to the value of the 2+ charge state.

16. Right-click **IDA Criteria** and then add another experiment. Change the **Scan type** to **Enhanced Product Ion**.
17. On the **MS** tab, type the parameters shown in [Table 1-11](#).



**Table 1-11 MS Tab Parameters**

Parameter	4000 QTRAP system	AB SCIEX QTRAP 5500 system
Mass Range	100 to 1500 m/z	100 to 1000 m/z
Number of scans to sum	2	1
scan speed	4000 Da/s	10000 Da/s

18. Click the **Advanced MS** tab and then set the parameters as shown in [Table 1-12](#).



**Note:** If you are using the AB SCIEX QTRAP 5500 system to analyze higher abundant proteins, you should select the Dynamic fill time feature instead of Q0 Trapping on the Advanced MS tab.

**Table 1-12 Advanced MS Tab Parameters**

Parameter	4000 QTRAP system	AB SCIEX QTRAP 5500 system
Scan mode	Profile	Profile
Step size	0.12	0.12
Resolution Q1	Unit	Unit
Fixed LIT fill time	25 msec	5 msec
Q0 Trapping	Yes	Yes

19. Click **Add experiment** and then add up to eight EPI experiments per cycle. Use the parameters from [Table 1-4](#) and [Table 1-5](#). The recommended number of EPI scans per cycle is one for the 4000 QTRAP system and three for the AB SCIEX QTRAP 5500 system.
20. Save the method.

