

8.1 PSD Quick Start

This section gives a quick overview of how to perform a PSD acquisition of angiotensin and an unknown in manual mode.

For detailed PSD information, refer to the sections that follow this PSD Quick Start.

Before you begin

Before starting this PDS Quick Start, be familiar with the information in:

- Section 8.2.1, Post-Source Decay Analysis
- Section 8.6.1, Observing the Effects of Laser Intensity
- Section 5.1, Loading, Modifying, and Saving Instrument Settings
- Section 6.2, Acquiring in Manual Mode from the Instrument Control Panel
- Section 6.4, Making Accurate Mass Measurements
- *Data Explorer User's Guide*, the sections that describe examining spectra and manual calibration

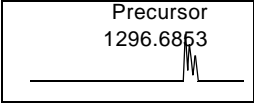

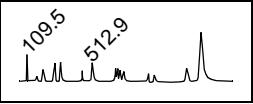

Two types of calibration in PSD analysis

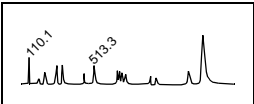
When you perform PSD analysis, you generate two types of mass calibration:


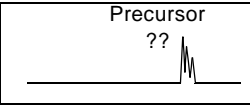
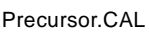
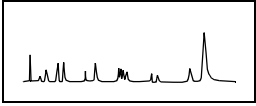
- **External calibration applied to precursor ion mass**— Normal mass calibration applied to a reflector mode spectrum, to ensure accurate mass of the precursor ion (described in Section 6.1.3, Calibrating the Mass Scale).
- **PSD calibration applied to fragment ion masses**— Special PSD calibration that optimizes fragment ion mass based on precursor ion mass and Mirror Ratio setting (described in Section 8.2.5, Mass Calculation for Fragment Ions).

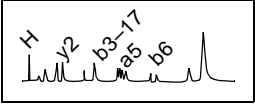
Steps to perform PSD analysis

The steps to perform PSD analysis on angiotensin and on an unknown are summarized in Table 8-1 and Table 8-2.

	Step	Result	See page
1.	Generate a Reflector mode precursor spectrum (use PSD_Precursor.BIC provided).	Angiotensin Reflector spectrum 	-6
2.	Generate a normal single-point external calibration using the spectrum acquired in step 1. You use this single-point external calibration in PSD analysis to obtain maximum mass accuracy for the precursor ion.	Normal external calibration for precursor ion mass accuracy 	-6
3.	Set PSD Acquisition/Instrument Settings parameters—Open Angiotensin_PSD.BIC provided, and: <ul style="list-style-type: none"> • In Instrument Settings—Type the angiotensin precursor mass and select Angio.CAL generated above (for precursor). • In PSD Acquisition Settings—Select default PSD calibration (for fragments). 	Angiotensin_PSD.BIC with: Angio.CAL Default PSD calibration	-6
4.	Acquire PSD segments (precursor and fragment spectra).	Angiotensin PSD Composite spectrum 	-8
5.	Generate a PSD multi-point external calibration using the spectrum acquired in step 4. You use this multi-point external calibration in PSD analysis to obtain maximum mass accuracy for the fragment ions.	PSD calibration for fragment ion mass accuracy 	-12


	Step	Result	See page
6.	<p>To verify that the Angio_PSD.CAL yields acceptable mass accuracy:</p> <ul style="list-style-type: none"> • Add the PSD calibration generated in step 5 to PSD Acquisition parameters— Open Angiotensin_PSD.BIC, select External PSD Calibration File, then select Angio_PSD.CAL (for fragments) • Acquire PSD segments (precursor and fragment spectra) with PSD calibration 	<p>Angiotensin_PSD.BIC with: Angio.CAL Angio_PSD.CAL</p> <p>Recalibrated angiotensin PSD Composite spectrum with optimum fragment ion mass accuracy</p> 	<p>-13</p> <p>-13</p>

	Step	Result	See page
1.	Generate a PSD calibration on angiotensin by performing step 1 through step 6 in "Steps to Perform PSD Analysis of Angiotensin" on page 8-3.	PSD calibration for fragment ion mass accuracy 	8-3
2.	Generate a precursor spectrum on the unknown in Reflector mode (use PSD_Precursor.BIC provided). NOTE: This step assumes that you have already determined an accurate mass for the precursor ion using reflector mode high-resolution analysis with internal or external calibration.	Unknown Reflector spectrum 	-6
3.	Generate a normal single-point external calibration using the spectrum acquired in step 2. You use this single-point external calibration in PSD analysis to obtain maximum mass accuracy for the unknown precursor ion.	Normal external calibration for precursor ion mass accuracy 	-6
4.	Set PSD Acquisition/Instrument Settings parameters—Open Angiotensin_PSD.BIC provided, and: <ul style="list-style-type: none"> • In Instrument Settings—Type the precursor mass and select Precursor.CAL generated in step 3 (for precursor). • In PSD Acquisition Settings—Select Angio_PSD.CAL generated in step 1 (for fragments). 	Angiotensin_PSD.BIC with: Precursor.CAL Angio_PSD.CAL	-6
5.	Acquire unknown PSD segments (precursor and fragment spectra) with Angio_PSD calibration.	Unknown PSD Composite spectrum with optimum fragment ion mass accuracy 	-13

	Step	Result	See page
6.	Optionally, confirm or investigate fragment ion identity using the Ion Fragment calculator or the Peptide Fragmentation macro.	Unknown PSD Composite spectrum with fragment ion labels 	-18

8.1.1 PSD Analysis of Angiotensin


Generating the Reflector mode precursor spectrum

1. Open the **PSD_Precursor.BIC** file provided with the software. This is a reflector mode instrument settings (.BIC) file. All other instrument settings should be identical to the settings in the PSD mode Angiotensin_PSD.BIC you use later to acquire segments.
2. Acquire using a laser intensity that does not saturate the reflector spectrum.
3. Save the precursor ion .DAT file by clicking  in the toolbar.

Generating an external calibration for the precursor ion

To obtain maximum mass accuracy for the precursor ion, follow the steps below to generate a single-point external calibration file to use when you perform the PSD acquisition.

NOTE: This is not a PSD calibration that affects fragment ion masses. It ensures accurate mass of the precursor.

1. Click  in the Instrument Control Panel toolbar to open the precursor ion data file in the Data Explorer software.
2. Create a single-point calibration using the precursor ion mass, then save the calibration file as **ANGIO.CAL** by exporting the calibration constants from the data file. For more information, see the *Data Explorer Software User's Guide*, Section 5.3.2, Manually Calibrating.

Setting PSD acquisition parameters

1. Open the **Angiotensin_PSD.BIC** file provided with the software. This is a PSD mode .BIC file.

The PSD Acquisition Settings control page (Figure 8-1) is automatically displayed in the Instrument Control Panel if you open a .BIC file set to PSD mode.

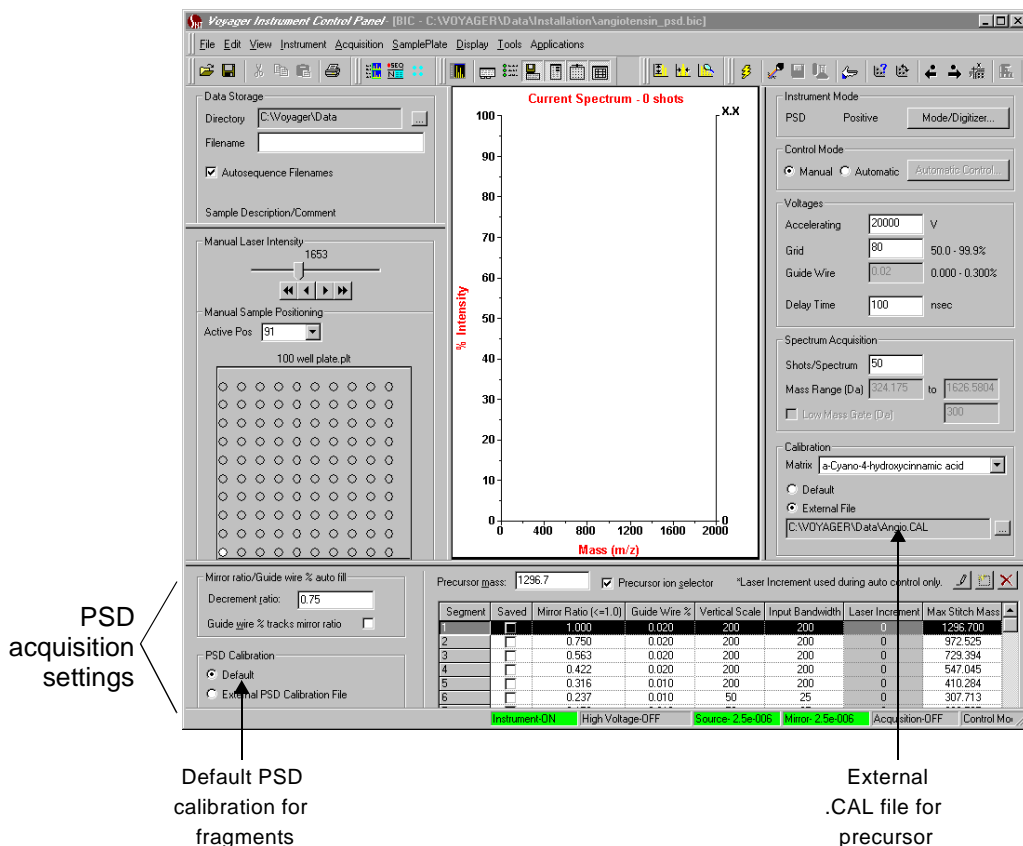


Figure 8-1 PSD Instrument Control Panel with Acquisition Settings Control Page

2. In the Calibration section of the Instrument Settings control page, select:

- The matrix you are using.
- **External File**, then select the **ANGIO.CAL** file you created in “Generating an external calibration for the precursor ion” on page -6.

3. In the PSD Acquisition Settings control page, type the angiotensin Precursor mass.

NOTE: Precursor mass is used for PSD calibration. Type in an accurate value with appropriate precision (for example, type 1296.68, not 1297).

4. Ensure that PSD calibration is set to **Default**.
5. Select **Save Instrument Settings As** from the File menu, then save the instrument settings file with a new name.

NOTE: The instrument settings files provided with your system (PSD_Precursor.BIC and Angiotensin_PSD.BIC) are read-only. You cannot save changes to these files unless you assign a new name.


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Acquiring PSD segments

1. In the Manual Laser Intensity/Sample Positioning control page, select the same sample position from which you acquired the precursor spectrum.
2. From the View menu, select **Data Storage**. Set parameters as needed. See "Setting Data Storage parameters" on page 6-14 for information.

Hint: Include a *_PSD* suffix when you name PSD data files to help you distinguish them from non-PSD data files. For example, if you type in *Experiment1_PSD* as the file name, the complete data file name will be *Experiment1_PSD.DAT* or *Experiment1_PSD_0001.DAT* (if Autosequence File Names is enabled).

Selecting and acquiring a segment

3. In the PSD Acquisition Settings control page, select the first row (click the number box in the Segment column) that corresponds to the segment you want to acquire.
4. To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .
5. Adjust laser intensity to optimize signal intensity. You typically need a higher laser intensity to optimize signal intensity for segments with lower Mirror Ratio settings.

The laser intensity needed for the first segment (the segment with the highest Mirror Ratio setting) is typically 150 to 200 counts higher than the laser intensity used to acquire the reflector mode precursor spectrum, and increases with each subsequent segment.

Examining and saving the segment

6. Examine the spectrum to ensure that fragments are produced (Figure 8-2).

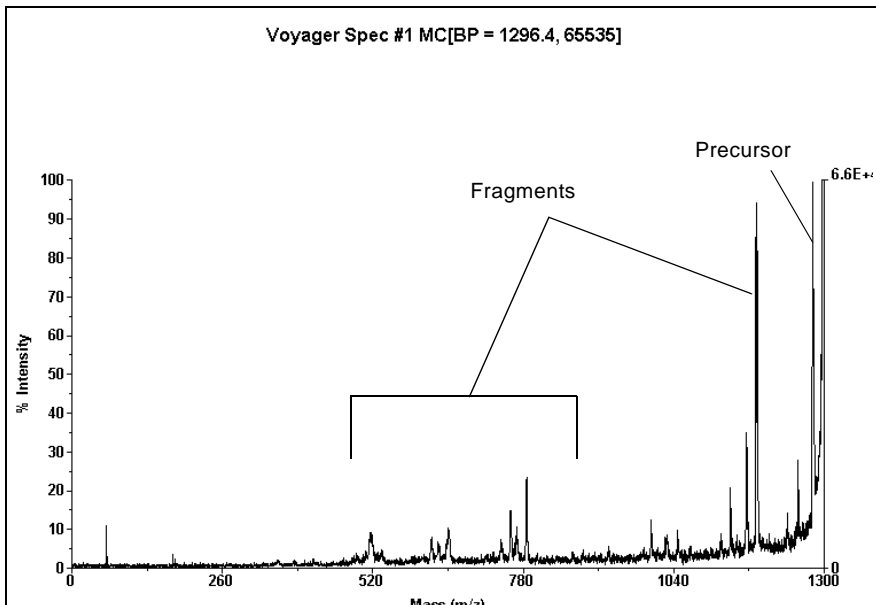



Figure 8-2 Segment Spectrum



7. If fragments are present and data is acceptable (Figure 8-2), click  in the toolbar to save the segment to the .DAT file. After you save the segment, the Saved check box in the segment list is checked.

If the current spectrum does not contain significant fragment ion signal and you do not want to save the spectrum, reselect the row and reacquire the spectrum, or select a new row.

CAUTION

Save the current segment (if the data is acceptable) before starting to acquire the next segment. If you do not, you will lose the data for the current segment.

Selecting and acquiring remaining segments

8. Repeat step 3 through step 7 to collect remaining segments.

NOTE: *Segments are listed in the Data Explorer software in the order in which they are acquired. If segments with duplicate Mirror Ratios are contained in the file, the software uses the last acquired segment when it generates the composite spectrum.*

Stopping the experiment


9. After you acquire all necessary segments, select **Stop Experiment** from the Acquisition menu. The PSD data file is closed.

You cannot view the PSD data file in the Data Explorer software until you stop the experiment.

CAUTION

If you stop an experiment without saving any segments, no .DAT file is created.

Determining if PSD calibration is needed

1. Click  in the Instrument Control Panel toolbar to open the PSD data file in the Data Explorer. The software “stitches” together portions of the fragment spectra and displays a composite spectrum (see Figure 8-6 on page 8-26 for an explanation of how the software generates the composite spectrum).

Your angiotensin composite spectrum should be similar to the spectrum shown in Figure 8-3.

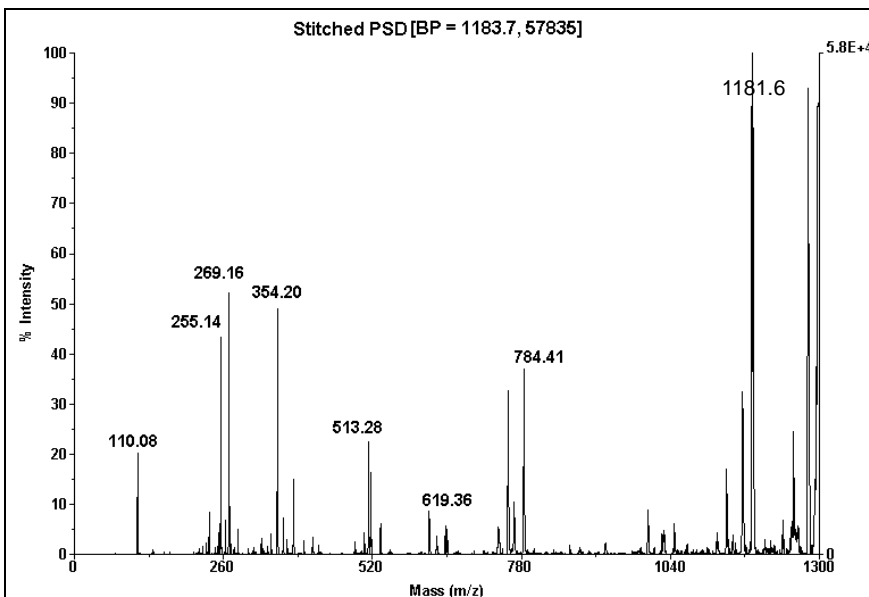


Figure 8-3 Angiotensin Spectrum

2. Examine the masses in the stitched spectrum and compare them to the expected masses listed in Table 8-1.

Table 8-1 Expected Masses in Angiotensin Spectrum

Expected Mass	Ion Type
110.08	His
255.16	b2–17
269.16	y2
354.20	b3–17
513.08	y4
619.36	a5
784.41	b6
1,181.6	y9

If masses are not within ± 0.2 Da (STR models) or ± 0.3 Da (PRO models) of the expected masses, generate a PSD calibration, and reacquire the PSD segments with the PSD calibration.

Generating a PSD calibration

This section gives a brief description of how to generate a PSD calibration. For more information, see the *Data Explorer User's Guide*, Section 8.3.3, Creating PSD Calibration (.CAL) Files and Applying to Other Data Files.

NOTE: This is a PSD calibration that affects fragment ion masses.

1. In the Data Explorer software, open the PSD data file acquired in the previous section, if it is not already open.
2. From the Process menu, select **Mass Calibration**, then select **PSD Calibration**.
3. Select the **Angiotensin_Fragments.REF** file provided with the software.

4. Select the peaks listed in Table 8-1 as reference masses for calibration by right-click-dragging on a peak, then select the correct mass for the peak in the Reference Peak dialog box.

For best mass accuracy:

- Select a minimum of seven reference masses.
 - Select peaks from different regions of the spectrum to ensure that high and low Mirror Ratios are represented.
5. Click **Solve and Plot** to generate the calibration, then click **Apply Calibration** to save the calibration constants in the data file. Click **Close**.
 6. Save the calibration file as **ANGIO_PSD.CAL** by selecting **Export** from the File menu, then selecting **Calibration**.

Adding PSD calibration

1. Open the Angiotensin_PSD.BIC file that you renamed in "Setting PSD acquisition parameters" on page -6.
2. In the PSD calibration section of the PSD Acquisition settings control page (see Figure 8-1 on page -7), select the **ANGIO_PSD.CAL** file you created in the previous section.
3. Select **Save Instrument Settings** from the File menu.


Acquiring PSD segments with PSD calibration

Reacquire PSD segments with PSD calibration as described in "Acquiring PSD segments" on page -8. Check the fragment ion masses to make sure they are within acceptable error.

8.1.2 PSD Analysis of an Unknown

Generating the Reflector mode precursor spectrum


This step assumes that you have already determined an accurate mass for the precursor ion using reflector mode high-resolution analysis with internal or external calibration (this analysis requires conditions that differ from PSD analysis conditions). You acquire the precursor ion again (using the same conditions you will use for PSD analysis) to generate a spectrum from which you can generate an external calibration. The external calibration you generate from the precursor ion is used to obtain maximum mass accuracy for the precursor ion during the PSD analysis, which helps ensure maximum mass accuracy for the fragment ions.

1. Open the **PSD_Precursor.BIC** file provided with the software. This is a reflector mode instrument settings (.BIC) file. All other instrument settings are identical to the settings in the PSD mode Angiotensin_PSD.BIC file you use to acquire segments.
2. Acquire using a laser intensity that does not saturate the reflector spectrum.
3. Save the precursor ion .DAT file by clicking  in the toolbar.

Generating an external calibration for the unknown precursor ion

To obtain maximum mass accuracy for the precursor ion, generate an external calibration file using the spectrum acquired in the previous section. You will use this external calibration when you perform the PSD acquisition. If you do not obtain maximum mass accuracy for the precursor ion during the PSD analysis, you will not obtain maximum mass accuracy for the fragment ions.

NOTE: *This is not a PSD calibration that affects fragment ion masses. It is an external calibration that is applied before PSD analysis to ensure accurate mass of the precursor.*

1. Click  in the Instrument Control Panel toolbar to open the precursor ion data file in the Data Explorer software.
2. Create a single-point calibration using the precursor ion mass and save the calibration file as **PRECURSOR_UNKNOWN.CAL** by exporting the calibration constants from the data file.

You may need to add the mass for the unknown to your calibration reference file before creating the single-point calibration.

For more information, see the *Data Explorer Software User's Guide*, Section 5.3.2, Manually Calibrating.

Setting PSD Acquisition parameters


1. Open the **Angiotensin_PSD.BIC** file provided with the software. This is a PSD mode .BIC file.
2. In the Calibration section of the Instrument Settings control page, select:
 - The matrix you are using.
 - **External File**, then select the **PRECURSOR_UNKNOWN.CAL** file you created for the unknown in "Generating an external calibration for the unknown precursor ion" on page -14.
3. In the PSD Acquisition Settings control page:
 - Type the accurate **Precursor mass**.
 - In the Calibration section, select **External File**, then select the he **ANGIO_PSD.CAL** file you created in "Generating a PSD calibration" on page -12.
4. Select **Save Instrument Settings As** from the File menu, then save the instrument setting file with a new name.

Acquiring PSD segments

1. In the Manual Laser Intensity/Sample Positioning control page, select the same sample position from which you acquired the precursor spectrum.
2. From the View menu, select **Data Storage**. Set parameters as needed. See "Setting Data Storage parameters" on page 6-14, for information.


Hint: Include a *_PSD* suffix when you name PSD data files to help you distinguish them from non-PSD data files. For example, if you type in *Experiment1_PSD* as the file name, the complete data file name will be *Experiment1_PSD.DAT* or *Experiment1_PSD_0001.DAT* (if Autosequence File Names is enabled).

Selecting and acquiring a segment

3. In the PSD Acquisition Settings control page, select the row (click the number box in the Segment column) that corresponds to the segment you want to acquire.
4. To start acquiring, select **Start Acquisition** from the Acquisition menu, or click  .
5. Adjust laser intensity to optimize signal intensity. You typically need a higher laser intensity to optimize signal intensity for segments with lower Mirror Ratio settings.

The laser intensity needed for the first segment (the segment with the highest Mirror Ratio setting) is typically 150 to 200 counts higher than the laser intensity used to acquire the reflector mode precursor spectrum, and increases with each segment.

Examining and saving the segment

6. Examine the spectrum to ensure that fragments are produced.
7. If fragments are present, click  in the toolbar to add the segment to the .DAT file. After you save the segment, the Saved check box in the segment list is checked.

If the current spectrum does not contain significant fragment ion signal and you do not want to save the spectrum, reselect the row and reacquire the spectrum, or select a new row.

CAUTION

Save the current segment (if the data is acceptable) before starting to acquire the next segment. If you do not, you will lose the data for the current segment.

Selecting and acquiring remaining segments

8. Repeat step 3 through step 7 to collect remaining segments.

NOTE: *Segments are listed in the Data Explorer software in the order in which they are acquired. If segments with duplicate Mirror Ratios are contained in the file, the software uses the last acquired segment when it generates the composite spectrum.*

Stopping the experiment

9. After you acquire all necessary segments, select **Stop Experiment** from the Acquisition menu. The PSD data file is closed.

You cannot view the PSD data file in the Data Explorer software until you stop the experiment.

CAUTION

If you stop an experiment without saving any segments, no .DAT file is created.

Confirming or investigating fragment ion identity

To confirm or investigate fragment ion identity, you can use the following tools:

- **If the peptide sequence is known**—Use the Ion Fragmentation calculator in the Data Explorer software to apply fragment labels to the unknown composite spectrum.

For information, see the *Data Explorer Software User's Guide*, Section 8.2, Applying Fragment Labels.

- **If the peptide sequence is not known**—Use the Peptide Fragmentation macro provided with the Data Explorer software to investigate the sequence in the unknown composite spectrum.

For information, see the *Data Explorer Software User's Guide*, Appendix C, Data Explorer Toolbox (Visual Basic Macros).