Isotopic Pattern Score Calculations

The TraceFinder application follows the same isotopic distribution logic as described in Isotopic Distribution in Exact Mass Spectra, but in a different order and with numerical limits and scores to optimize automatic processing. After the TraceFinder application determines the possible elemental compositions for a particular compound of interest, it calculates a expected isotope pattern for each elemental composition candidate and an isotopic pattern score to represent the fit between the expected and measured isotope patterns.

The following example describes the isotopic pattern score data and provides the score calculation details for one specific data set.

Data Set Example

For this example, use the "Pesticides Screening Example" data set installed in the …\TraceFinderData folder. In the Data Review view, select the **Apple_PosHCD__4O_5_O1** sample and the **Metribuzin** target compound.

For this compound, note that the Isotopic Pattern Score column shows 90% and the Num Isotopes Matched column shows "2 of 3" in the Compounds pane in the Data Review. The compound's formula is C8H14N4OS and its adduct is H, so the modeled isotopic pattern is C8H15N4OS.

Isotopic data for Metribuzin

	Selected	Compound Name	Formula	Isotopic Pattern Score (%)	Num Isotopes Matched
	Aa ▾	Aa ▾	Aa ▾	= ▾	Aa ▾
640	☐	Metribuzin	C8H14N4OS	90	2 of 3

In the Spectrum pane, click the Isotopes tab and zoom in to view the expected isotopic pattern spectrum compared to the acquired, measured spectrum. The resulting isotopic pattern score should correlate to a visual inspection of the difference between the expected isotope display and the measured display for the target compound.

Isotopic pattern spectra (stacked)
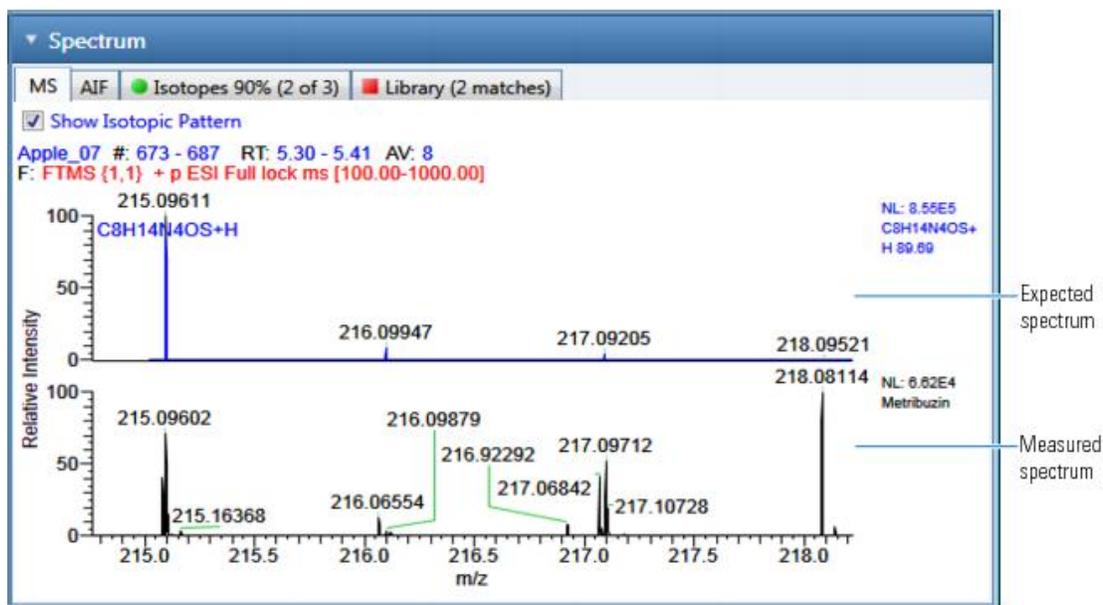
For this example, the processing method used to process the data contains the following isotopic pattern settings for target screening:

- Fit threshold = 90%

- Allowed Mass Deviation = 5 ppm

- Allowed Intensity Deviation = 10%

- Use Internal Mass Calibration = Cleared

The data for the **Metribuzin** compound is as follows:

Measured	Expected
A0 *m/z* = 215.09602	A0 *m/z* = 215.09611
A0 Noise = 1482	–
A0 intensity = 4.75E4	A0 intensity = 8.55E5

Because the measured and the expected spectra have different intensities, the spectral noise threshold must proportionally apply to the expected spectrum to decide which peaks are expected in the measured data.

Noise threshold (expected) = *Noise of A0 (measured)* $\times$ *Intensity of A0 (expected)* $\div$ *Intensity of A0 (measured)*

Following the previous formula, the expected noise threshold is 1482 $\times$ 8.55E5 $\div$ 4.75E4 = 2.6676E4.

The expected ions in the measured spectrum are those whose intensities are above the expected noise threshold. The following tables lists the ions in the expected spectrum. A "3" in the Above Threshold column indicates the expected ions.

Ions in expected spectrum

Isotope order	*m/z* (expected)	Intensity (expected)	Above threshold	Present in measured spectrum
A0	215.09611	8.55E5	3	Yes

A1	216.09945	7.50E4	3	Yes
A2	217.09191	3.86E4	3	Yes
A3	218.09521	3.39E3		No

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Calculating Mass and Intensity Deviations

The expected number of ions is 3, as shown by those ions with a "3" in the Above Threshold column of the Ions in expected spectrum table. These are the ions you focus on for the scoring calculations. This number "3" shows as the value of y in the Num Isotopes Matched column of the Compounds pane in the Data Review. It indicates the number of expected isotopic pattern peaks based on the Fourier transform (FT) noise in the spectrum.

In this case, you expect to see the three most intense expected peaks in the measured spectrum. The masses of those peaks are (in order of intensity from high to low): 215.09611, 216.09945, and 217.09191. When the measured spectrum is more intense or the noise level is lower, you find more peaks passing the noise threshold and expected in the measured spectrum, eventually including other isotopic peaks.

The following table shows the mass deviation (delta m/z) data for each of the expected ions.

Mass deviation data for the expected ions

Isotope order	m/z (expected)	m/z (measured)	Delta m/z (ppm)
A0	215.09611	215.09602	−0.42
A1	216.09945	216.09879	−3.05
A2	217.09191	217.09712	24

where

Delta m/z (ppm) = $1\,000\,000 \times ([m/z \text{ (measured)} - m/z \text{ (expected)}] \div m/z \text{ (expected)})$

For example:

$1\,000\,000 \times ([216.09879 - 216.09945] \div 216.09945)] = -3.05$ ppm

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Tip   You can see the expected, measured, and delta m/z values on the Isotopes page of the Spectrum pane. The MS page (see Isotopic pattern spectra (stacked)) displays the profile measured m/z values, whereas the Isotopes page displays the centroid measured m/z values, which might be different.

If you want more precision, you can see extra decimal digits for the A0 m/z values in an exported data file.

If the absolute value of the Delta m/z is less than 5 ppm (the Allowed Mass Deviation value set in the processing method), the TraceFinder application determines that this ion is found—that is, the ion is present in the measured spectrum. For this data set

example, the application finds only the A0 and A1 ions, so "2" shows as the value of x in the Num Isotopes Matched column of the Compounds pane in the Data Review. The application does not find the A2 expected ion because the absolute value of its Delta m/z of 24 ppm is much higher than 5 ppm.

Note You can see from zooming in on the isotopic pattern spectra (see Isotopic pattern spectra (stacked)) that there are measured peaks in the measured spectrum closely corresponding to the first two expected ions, but there is not a measured peak closely corresponding to the 217.09191 expected ion.

The following table lists the intensity deviation (delta intensity) data for each of the expected ions, relative to the A0 ion's expected intensity of 8.55E5 and measured intensity of 4.75E4.

Intensity deviation data for the expected ions

Isotope order	m/z (expected)	Intensity (expected)	Relative intensity (expected, %)	Intensity (measured)	Relative intensity (measured, %)	Delta intensity
A0	215.09611	8.55E5	100	4.75E4	100	0
A1	216.09945	7.50E4	8.77	8.75E3	18.42	9.65
A2	217.09191	3.86E4	4.51	3.47E4	73.05	68.54

where

- Relative intensity (expected and measured) values are derived from the isotopic pattern spectra (see Isotopic pattern spectra (stacked)). Each value is a percentage of the isotope's intensity relative to the A0 ion's intensity.

  For example: 7.50E4 ÷ 8.55E5 = 8.77%

- Delta intensity = *Relative intensity (measured) – Relative intensity (expected)*

  For example: 18.42 – 8.77 = 9.65

In this example, the absolute values of the Delta m/z for the A0 and A1 ions (see Mass deviation data for the expected ions) are both less than the Allowed Mass Deviation of 5 ppm; therefore, the application considers that these two ions are present in the measured spectrum. The delta intensity of the A1 isotope ion is close to the Allowed Intensity Deviation of 10% and the delta intensity of the A2 isotope ion is much higher (see Intensity deviation data for the expected ions).

The TraceFinder application determines the isotopic pattern score value from a combination of the mass and intensity deviations between the expected and the measured spectra. In this case, the application reduces the isotopic pattern score value down to 90% from 100% to reflect the marginal quality of the intensities of the A1 and A2 isotopes and to penalize for not finding the A2 isotope.


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Calculating Isotopic Pattern Score

To score the fit for an isotopic pattern, the TraceFinder application calculates each expected ion's fit and then combines the individual fit scores, weighted by their expected intensities.

For each expected ion peak, the application measures the m/z and intensity differences between the expected and the measured patterns. It then normalizes those differences (normalized deviation values) to the maximum allowed mass and intensity deviation

values set in the processing method. The application then sums the normalized differences by vector addition (see Vector sum of intensity (I) and mass (M) deviations).

Measured and expected patterns
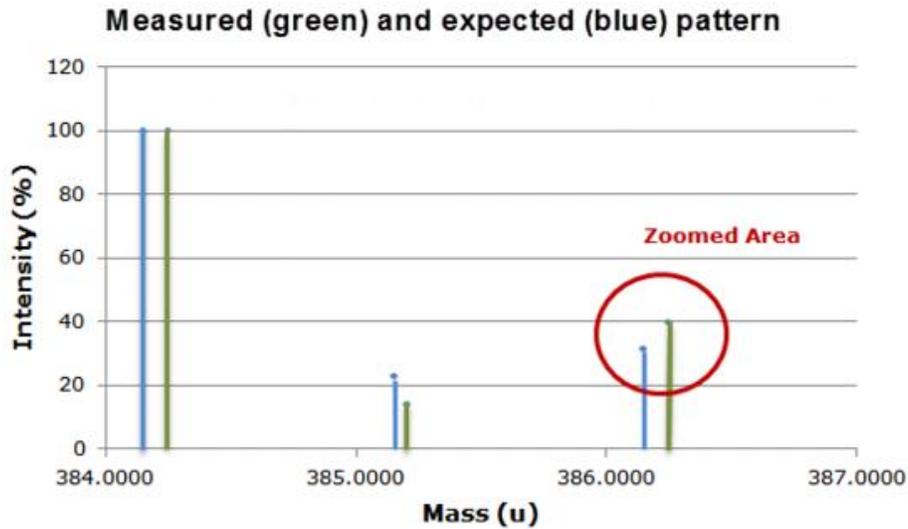
Vector sum of intensity (I) and mass (M) deviations
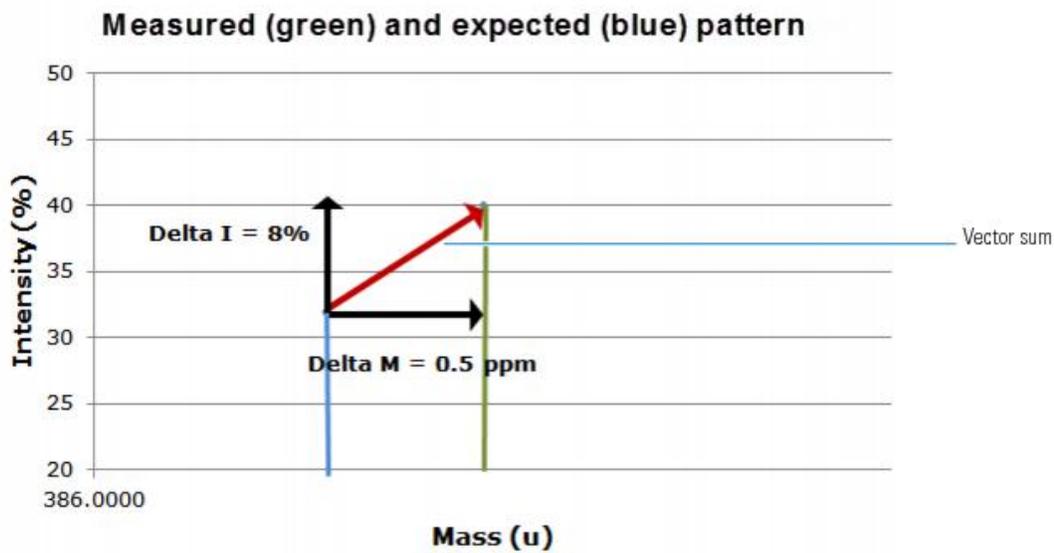
This example starts first with the intensity deviations. The Allowed Intensity Deviation value set in the processing method is 10%, so this is the normalization value. As shown in Intensity deviation data for the expected ions, the delta intensity value for the A1 isotope is close to 10%, resulting in a normalized intensity deviation close to 1.0.

Normalized Intensity Deviation

The following table lists the normalized intensity deviation data for each of the expected ions.

Normalized intensity deviation data for the expected ions

Isotope order	m/z (expected)	Delta intensity	Allowed intensity deviation	Normalized intensity deviation

		(%)		
A0	215.09611	0	10	0 ÷ 10 = 0.0
A1	216.09945	9.65	10	9.65 ÷ 10 = 0.965
A2	217.09191	68.54	10	68.54 ÷ 10 = 6.854

Next are the mass deviations. For mass deviations, you can control two settings in the processing method:

- The first setting is the Allowed Mass Deviation that functions as an outer limit in the same way as the Allowed Intensity Deviation functions as a limit for the intensity.

- The second setting is the Use Internal Mass Calibration check box. If you do not select this check box in the method, then the application considers any mass value within 2 ppm of the expected m/z as a perfect match (no deviation). If you select this check box, then the application considers only a mass value within 1 ppm of the expected m/z as a perfect match.

In this example, the Allowed Mass Deviation value set in the processing method is 5 ppm and the Use Internal Mass Calibration check box is cleared. The mass normalization is a bit more complex than the intensity normalization because mass values < 2 ppm (Use Internal Mass Calibration setting) from the expected m/z are considered to have no deviation from theory; however, for values between 2 ppm and 5 ppm (Allowed Mass Deviation value) from the expected m/z, the normalized deviation varies from 0 to 1.

The calculated normalized mass deviation value is as follows:

- 0 if absolute value (*Delta m/z*) < 2 ppm

  where 2 ppm is the value from the Use Internal Mass Calibration setting.

- [absolute value (*Delta m/z*) − 2 ppm] ÷ (5 ppm − 2 ppm) if absolute value (*Delta m/z*) ≥ 2 ppm

  where 2 ppm is the value from the Use Internal Mass Calibration setting and 5 ppm is the Allowed Mass Deviation value.

In this case, the absolute value of the mass deviation for the A0 ion is less than 2 ppm; therefore, its normalized mass deviation value is 0.

Normalized Mass Deviation

The following table lists the normalized mass deviation data for each of the expected ions.

Normalized mass deviation for each of the expected ions

Isotope order	m/z (expected)	m/z (measured)	Delta m/z (ppm)	Internal calibration (ppm)	Allowed mass deviation (ppm)	Normalized mass deviation
A0	215.09611	306.10356	−0.42	2	5	0
A1	216.09945	307.10691	−3.05	2	5	0.35
A2	217.09191	308.11324	24	2	5	7.33

For example: [(24 − 2)] ÷ (5 − 2) = 7.33

To calculate the combined deviations, the application uses the Pythagorean theorem to calculate the vector sum of the normalized deviations. The calculation for the vector sum is as follows:

Vector sum = Square root [(*Normalized intensity deviation*)^2 + (*Normalized mass deviation*)^2].

However, if the vector sum > 1 then set it to 1.

Calculated Vector Sum

The following table lists the vector sum data for each of the expected ions.

Calculated vector sum

Isotope order	m/z (expected)	Normalized intensity deviation	Normalized mass deviation	Vector sum
A0	215.09611	0	0	0
A1	216.09945	0.965	0.35	1
A2	217.09191	6.854	7.33	1

To calculate the final score, you must weigh the vector sum values and then express the result as a percentage value. Each ion's weighting contribution to the final isotopic pattern score is proportional to its intensity.

Weighting Factor Calculations

The following table lists the weighting factor of each of the three expected ions.

Weighting factor calculations

Isotope order	m/z (expected)	Intensity (expected)	Weighting factor for final score
A0	215.09611	8.55E5	0.8827
A1	216.09945	7.50E4	0.0774
A2	217.09191	3.86E4	0.0399
		Sum = 9.686E5	Sum = 1.000

The weighting factor of each individual ion = *Intensity of each ion ÷ Sum of intensities of all expected ions*

For example: 8.55E5 ÷ 9.686E5 = 0.8827

When not all of the expected ions are present in the measured spectrum, the application applies a penalty value (1, 2, or 4) to the weighted deviation of each missing ion, lowering the final isotopic pattern score even further. The penalty value depends on how strong the ion signal is expected to be in the measured spectrum. For the A2 ion that is not found, the application sets its penalty to a value of 1, causing its vector sum value of 1 in this table to be replaced with the penalty value of 1 in this table. In this case, it is the same number, but for other cases, the penalty value might be different from the vector sum value.

Calculated Isotopic Pattern Score

The following table lists the calculated isotopic pattern score using the weighting factors.

Calculated isotopic pattern score

Isotope	m/z	Deviation (vector sum or	Weighting factor	Weighted

order	(expected)	penalty)		deviation
A0	215.09611	0 (vector sum)	0.8827	0
A1	216.09945	1 (vector sum)	0.0774	0.0774
A2	217.09191	1 (penalty)	0.0399	0.0399
				Sum = 0.12

where

- Weighted deviation of each individual ion = *Deviation* ✕ *Weighting factor*

  For example: 1 ✕ 0.0774 = 0.0774

- Isotopic pattern score = 100% ✕ (1.0 – *Sum of all weighted deviation values*)

  For this example, the calculated isotopic pattern score of 100% ✕ (1.0 – 0.12) is 88%, which is close to the 90% score displayed in the application.

Note Use these calculations to approximate the score displayed in the application. The calculated score might not match exactly the score in the application because some internal calculation details are not listed here or there is discrepancy due to decimal digit rounding.

In certain cases, closely matching isotopes exist because heavier isotopes contribute to the isotopic pattern observed in the mass spectra. For example, the isotopes 206.0941 and 206.1006 result from the contribution of one heavier isotope of carbon and one heavier isotope of nitrogen, respectively, together making up the split A1 isotopic peak. When the application performs isotopic pattern scoring, this situation appears as a main isotopic peak with a smaller peak to the side whose *m/z* is included in the calculations.


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Finding the Noise Value

❖ To find the noise value associated with a mass spectral peak

1. On the Isotopes page of the Spectrum pane, to view the expected spectrum stacked above the measured spectrum, right-click the spectrum plot area and choose **Display Stack Spectra** from the shortcut menu.

2. Zoom in to the peak of interest.

   As an example, see Isotopic pattern spectra (stacked) for the compound **Metribuzin**.

3. To view the averaged noise value (N) for a peak in the measured spectrum, right-click the spectrum plot area and choose **Show Noise Label** from the shortcut menu.
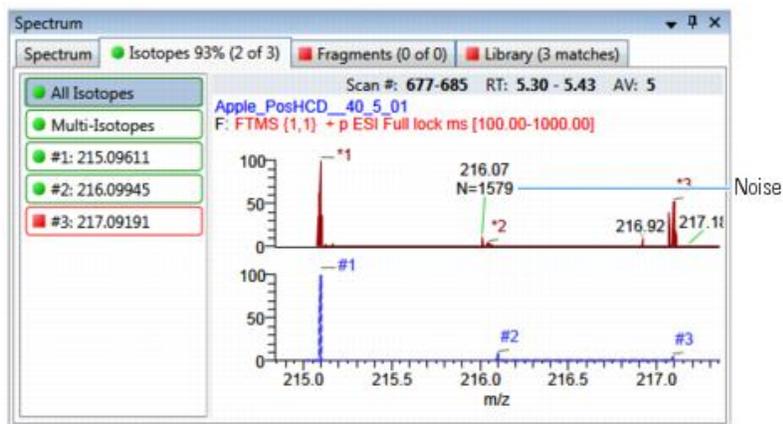
In this example, the averaged noise value for the peak is 1579.