# Signal-to-Noise Values in Empower 3

This Technical Note describes how to calculate Signal-to-Noise (s/n) Ratio in Empower 3. There are four different methods that you can use to calculate the signal-to-noise ratio.

- Calculating signal-to-noise using noise from within the same chromatogram
- Calculating signal-to-noise using noise from a blank injection
- Calculating signal-to-noise in non-pharmaceutical or non-regulated environments
- Calculating signal-to-noise using a custom field

#### **Definition of terms**

### Height

Height is defined as measured from the apex of the peak to the baseline of the peak. Peak height is reported in microvolts ( $\mu$ V).

### Peak-to-peak noise

Peak-to-Peak Noise is the noise value that Empower uses to calculate EP, JP, or USP s/n. Peak to Peak Noise is defined to be the algebraic difference of the maximum and minimum *residuals* between each data point and the least-square line. The *residual* is determined by subtracting the y value of the data point predicted by the line from the y value of the data point.

The formula for Peak to Peak Noise is:

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Peak to Peak Noise = max residual - min residual
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where:

residual = (yi - ypi)

*yi* = Value of the data point

ypi = Value of the data point predicted by the line

Peak to Peak Noise is expressed in detector units. For example, the peak to peak noise calculation for a UV detector would be expressed in absorbance units (AU).

Average Peak to Peak Noise is calculated by dividing the data into segments (specified in the processing method) and averaging the values for each segment.

In the example shown in Figure 1, the Peak to Peak Noise is the sum of both maximum residual  $(1.07 \times 10^{-6})$  and minimum residual  $(6.57 \times 10^{-7})$ , which is  $1.72 \times 10^{-6}$ .



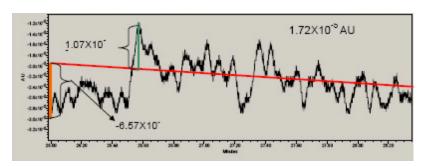


Figure 1 – Peak-to-peak noise determination

Signal-to-Noise definition based on USP <621> Chromatography Chapter (reference USP 38 NF 33, effective 5/1/2015 to 4/30/2016)

S/N is calculated as follows:

$$S/N = \frac{2H}{h}$$

where:

H = Height of the peak (Figure 2) measured from the peak apex to a baseline extrapolated over a distance  $\geq 5$  times the peak width at its half-height.

h = Difference between the largest and smallest noise values observed over a distance
 ≥ 5 times the width at the half-height of the peak and, if possible, situated equally around the peak of interest.

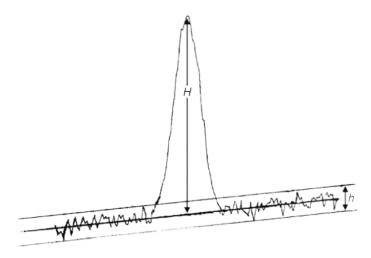


Figure 2 – USP signal-to-noise determination

## Signal-to-Noise definition based on EP 8.8

S/N is calculated as follows:

$$S/N = \frac{2H}{h}$$

where:

H = Height of the peak (Figure 3) corresponding to the component concerned, in the chromatogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to at least 5 times the width at half-height.

h = Range of the noise in a chromatogram obtained after injection or application of a blank, observed over a distance equal to at least 5 times the width at the half-height of the peak in the chromatogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

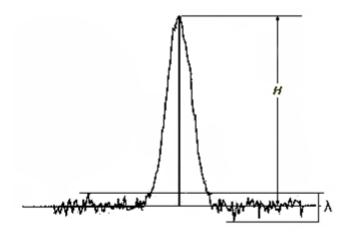


Figure 3 – EP signal-to-noise determination

### Difference of Height measured in Empower versus how USP or EP defines Height

There is a difference in how Empower measures peak height as compared to how it is defined in the Pharmacopoeias (Figure 4).

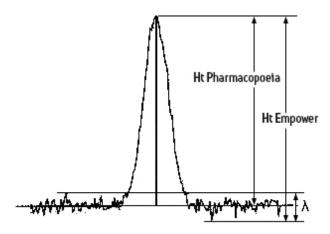


Figure 4 – Height comparison between Empower and Pharmacopoeia

- Ht Pharmacopoeia Peak height from the peak apex to the middle of the noise as seen in the diagrams presented by the Pharmacopoeias (USP, EP, and JP).
- Ht Empower Height measured from the peak apex to the lowest signal point in the baseline and is reported in microvolts (µV). This is true for both ApexTrack and Traditional peak detection and integration.

The difference between Ht Empower and Ht Pharmacopoeia is ½ Peak to Peak Noise. Due to this difference, Empower calculates signal to noise (USP s/n, EP s/n and JP s/n) based on the following formula.

Empower S/N = 2 x (Height - (0.5 x Noise / Scaling)) / (Noise / Scaling)

where:

Height = Empower field that is measured from peak apex to the lowest signal point in the baseline.

Noise = Represented as the Empower peak fields USP Noise, EP Noise and JP Noise if using a blank injection or the Empower result field Peak to Peak Noise if calculated using a section of the same chromatogram.

Scaling = Scale to  $\mu V$  where it allows conversion of Plot units to Height ( $\mu V$ ) units. In an example where you have a UV detector where the Plot Units is AU, the Scale to  $\mu V$  is 1 x 10<sup>-6</sup> Plot Units/ $\mu V$ .

### **NOTES:**

- 1. You must have the System Suitability option installed and enabled in the project for which you want to set suitability limits.
- 2. By default, the software reports the EP, JP, or USP s/n value to a precision of 6, not in scientific notation, and with no units.

# Historical development of Empower s/n calculations

When Empower 2 was released in May 2005, USP had not yet defined any s/n calculations. With USP 31 NF 26 (effective May 1, 2008 - April 30, 2009), there was no s/n formula in the guidelines. However, beginning with USP 32 NF 27 (effective May 1, 2009 – April 30, 2010), USP defined the s/n formula as 2H/n. Because of this update, Waters released Technical Note TECN10123052 Rev 00 in September 2009 that included suggested custom fields that you could use with or without a blank injection.

In both occasions, noise was measured via the Noise and Drift tab by specifying a fixed region of choice. With these custom fields, Empower 2 allowed height to be measured from the apex of the peak to the bottom of the noise in the baseline. In comparison, the built-in EP s/n value was calculated by subtracting half the noise to allow Height to be determined from the apex of the peak to the middle, rather than the bottom, of baseline noise. Based on these calculations, there was a difference in the EP s/n by a value of 1 when you used the custom fields suggested in the Technical Note.

With USP 34 NF 29 (effective date May 1, 2011 – April 30, 2012), USP provided a better description of their formula by adding a diagram (Figure 2) together with the s/n formula. This clarified that the height measurement needed to be adjusted by half the noise, just as Waters had been doing in Empower 2 with the built-in EP s/n calculation.

These changes were released in Empower 3 software when it was newly released in January 2011. In Empower 3, the built-in USP s/n field calculation accommodates not only the compensation of subtracting half the noise but also allows the half height multiplier to be effective in adjusting where to measure Peak-to-Peak Noise on an identified blank injection. If you do not use a blank injection, Empower 3 still uses Noise and Drift with a fixed Start and Stop time as to where to measure Peak-to-Peak Noise.

Since Empower 3 was released, the built-in field has been used widely by our customers. Until the current release of USP 38 NF 33, and with upcoming release of USP 39 NF 34, the s/n formula has not been modified.

In response to USP 35 chapter <621>, Chromatography, effective 01 December 2012, as well as changes to Chapter 2.2.46, Chromatographic Separation Techniques, of EP 7.06, the Empower 3 signal-to-noise calculation was modified effective with Empower 3 Feature Release 2 (FR2):

- USP s/n is still calculated as 2H/h, but the determination of H and h has been changed to match the EP and JP s/n calculations. The default width of the region used to calculate h (the half height multiplier) remains at 5.
- EP s/n is also still calculated as 2H/h, but the default width of the region used to calculate h (the half height multiplier) is now 5 instead of 20.

# Calculating the signal-to-noise ratio

#### Calculating the signal-to-noise ratio from within the same chromatogram

You can calculate USP, EP, and JP signal-to-noise ratios using the same chromatogram without having to make blank injections in the same sample set or without the need to create a custom field.

1. In the Suitability tab of the processing method, select Calculate USP, EP, and JP s/n and then deselect Use noise centered on peak region in blank region (Figure 5).

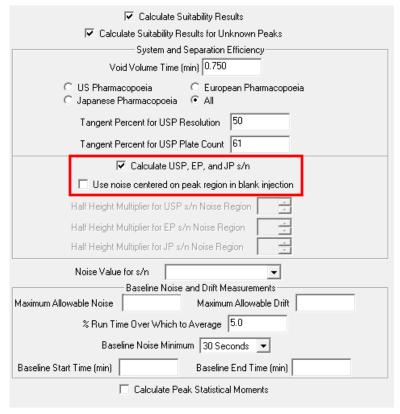


Figure 5 – Processing Method Suitability tab, calculating s/n from the same chromatogram

2. Select the **Noise and Drift** tab. A warning message may appear if the Noise and Drift tab has not been set up. Click **Yes** to continue to the Noise and Drift tab (Figure 6).

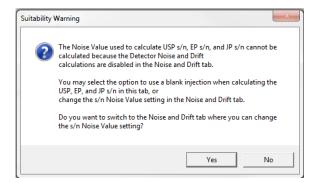


Figure 6 – Suitability Warning message

- 3. In the Noise and Drift tab, select **Calculate Detector Noise and Drift** (Figure 7). Selecting this option allows the following fields to be calculated: Detector Noise, Average Detector Noise, Peak to Peak Noise, and Average Peak to Peak Noise. These fields are available in the Results window of Review.
- 4. Next, specify the **Start and Stop Times** of the region in the chromatogram where there are no peaks of interest.
- 5. Finally, specify a **Segment Width**, although this is only relevant for the calculations that are Average Peak to Peak Noise and Average Detector Noise.

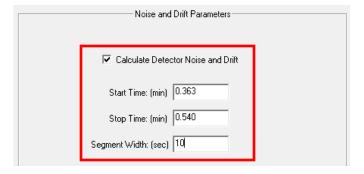


Figure 7 – Noise and Drift Parameters

Figure 8 shows example results when Peak to Peak Noise is used to calculate USP s/n, EP s/n, and JP s/n.

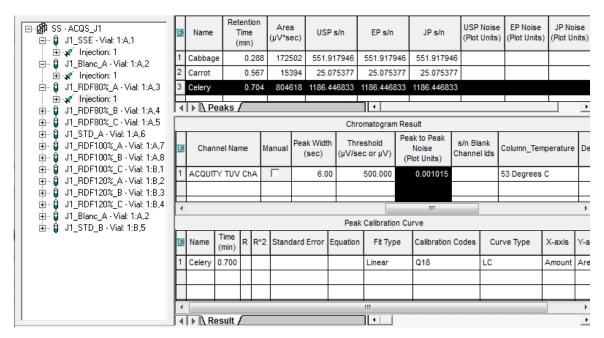


Figure 8 - Example results when using the same chromatogram to calculate the s/n values

### Calculating the signal-to-noise ratio using a blank injection

You can calculate USP, EP, and JP signal-to-noise ratios and have the Peak to Peak Noise calculated from one or more blank injections in the same sample set as follows:

1. In the Suitability tab of the processing method, select Calculate USP, EP, and JP s/n and then select Use noise centered on peak region in blank region (Figure 9).

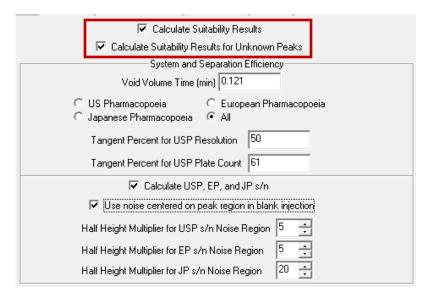


Figure 9 – Processing Method Suitability tab, using a blank injection

When this option is selected, the software calculates a noise value for each peak using the peak-to-peak noise determined in the blank injection. The value is calculated for a region in the same channel in a separate blank injection. This region is centered around the peak's retention time and has a width equal to the peak's width at half height (width at 50% height) x the Half Height Multiplier for EP, JP, or USP Noise Region value.

The software reports this noise value in the result as USP Noise, EP Noise and JP Noise to a precision of 6 (the default), not in scientific notation, and using the *units* Plot Units. The default Half Height multipliers are listed below; you can modify these values as desired.

Selected Pharmacopoeia	Half Height Multiplier Default Value
USP	5
EP	5
JP	20

**NOTE:** In Empower 3 Feature Release 2 (FR2), which was based on EP 7.06, the half height multiplier was changed to 5. This means that USP s/n and EP s/n are now calculated the same way.

You can calculate USP, EP, and JP signal-to-noise ratios using one or more blank injections in the same sample set without needing to create a custom field. The method Empower uses to calculate signal-to-noise using a blank value depends on the number of blank injections:

No. of Blank Injections	Calculation Method
Single blank injection	Determines the peak-to-peak noise value for each peak in the blank injection
Multiple blank injections	Determines the peak-to-peak noise value for each peak in the blank injections, averages them, and then uses the average noise value

The s/n Blank Channel IDs field displays the IDs of the blank channels used to determine USP, JP, and EP noise, and subsequently USP, JP, and EP s/n.

**NOTE:** The blank channel must appear earlier in the sample set method than the channels that use it to calculate USP s/n, EP s/n, and JP s/n. The noise region in the blank requires a minimum of five data points to determine noise. Ten or more are recommended.

2. For each blank injection in the sample set, in the Run Samples, Sample Set Method editor, or the Alter Sample table, select the **Blank** check box.

**NOTE:** You also use the Blank check box for custom field formulas. When you calculate signal-to-noise with the **Use noise centered on peak region in blank injection** option selected, the noise value is either a noise value calculated from a single blank channel or an averaged noise value calculated from multiple blank channels. The new suitability result field s/n Blank Channel IDs displays the IDs (separated by spaces) of the blank channels used. When USP s/n, JP s/n, and EP s/n are not calculated using noise values from blank channels, the s/n Blank Channel IDs field is empty.

If you choose not to apply the noise value from one or more blank injections, the software uses the peak-to-peak noise value, according to the parameters specified in the processing method's Noise and Drift tab. Empower uses the Peak-to-Peak Noise field as the noise value for USP s/n, EP s/n, and JP s/n only if you select the **Calculate USP**, **EP**, and **JP** s/n check box, and deselect the **Use noise centered on peak region in blank injection** check box.

Figure 10 shows example results for USP s/n, EP s/n, and JP s/n, and the Noise values USP Noise, EP Noise, and JP Noise based on a blank injection in Vial 1:A,2.

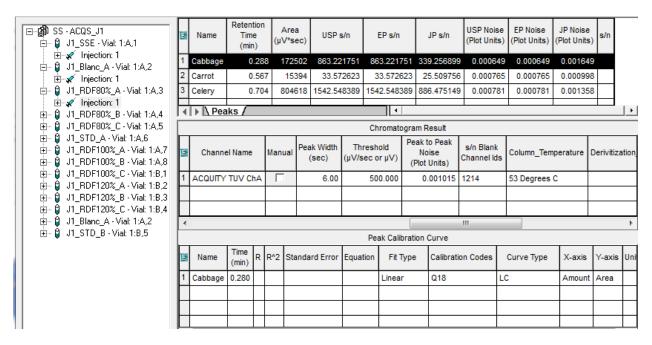


Figure 10 - Example results when using a blank injection to calculate the s/n values

#### Calculating the signal-to-noise ratio in a non-pharmaceutical environment

In a non-pharmaceutical or non-regulated environment, you can calculate signal-to-noise ratios using Empower's built-in s/n field. This field has been available since Empower 2 was released.

Empower's built-in s/n field uses the following formula:

Empower s/n = height / (noise/scaling)

where:

Height = Empower field that is measured from peak apex to the lowest signal

point in the baseline.

Noise = The result's user-specified noise value in the Noise Value for s/n

field.

Scaling = Scale to convert the Noise Value to  $\mu V$  (for example, scaling = 1 x

10<sup>-3</sup> if the Noise value for s/n is Baseline Noise (mV)

To calculate the signal-to-noise ratios using Empower's built-in s/n field:

1. In the Suitability tab of the processing method, select the desired noise value from the **Noise Value for s/n** drop-down list (Figure 11). The choices are Detector Noise, Average Detector Noise, Peak to Peak Noise, Average Peak to Peak Noise, or Baseline Noise.

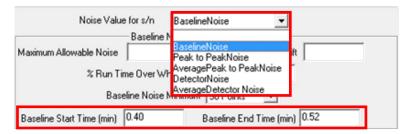


Figure 11 - Noise Value for s/n choices

- 2. If you select **Baseline Noise**, enter a **Baseline Start Time** and **Baseline End Time** in the Baseline Noise and Drift Measurements section directly below the drop-down list.
- 3. If you select any of the other noise values, proceed as follows:
  - a. In the Noise and Drift tab, select Calculate Detector Noise and Drift (Figure 7). Selecting this option allows the following fields to be calculated: Detector Noise, Average Detector Noise, Peak to Peak Noise, and Average Peak to Peak Noise. These fields are available in the Results window of Review.
  - b. Next, specify the **Start and Stop Times** of the region in the chromatogram where there are no peaks of interest.
  - c. Finally, specify a **Segment Width**, although this is only relevant for the calculations that are Average Peak to Peak Noise and Average Detector Noise.

The following table describes the available noise value selections in the Noise Value for s/n drop-down.

Noise value	Description	
Detector noise	The root mean square (RMS) noise of the data is calculated using the least-squares line. The formula for Detector Noise is:	
	Detector Noise = $((sum(yi - ypi))^2 / (n-2))^{\frac{1}{2}}$	
	where:	
	yi = The y value of the data point	
	ypi = The y value of the data point predicted by the line	
	n = Number of data points	
	<b>NOTE:</b> (n-2) is used as the denominator in the above calculation because a linear fit is used (and 2 coefficients are required).	
	Detector Noise is expressed in detector units. For example, the noise calculation for a UV detector would be expressed in absorbance units (AU).	
	Average Detector Noise is calculated by dividing the data into segments (specified in the processing method) and averaging the values for each segment.	

Noise value	Description		
Detector noise	Detector Noise is expressed in detector units. For example, the noise calculation for a UV detector would be expressed in absorbance units (AU).		
(continued)	Average Detector Noise is calculated by dividing the data into segments (specified in the processing method) and averaging the values for each segment.		
	A least-squares (best fit) line is fitted to the whole time interval (specified in the processing method) of the data and is used to perform all calculations. Typically, the formula for a line is $y = mx + b$ . However, in Empower, the formula for a line is:		
	y = a + bx		
	where:		
	a = The y-axis intercept		
	b = The slope of the line		
	Many of the calculations compare the actual value of the data point and the predicted value of the data point based on the least-squares line.		
	Figure 12 shows a visual representation of the least-squares line:		
	Value of data point		
	0.0000		
	0.00002		
	Value of data point		
	0.50000		
	4,00 4,10 4,20 4,30 4,40 4,50 4,70 4,50 4,90 5,00 Meules		
	Figure 12 – Representation of the least-square line		
Peak-to-peak noise	Refer to the Peak-to-peak noise description on page 1.		
Baseline noise	Short term noise is noise resulting from detector electronics such pump pulsations. Baseline Noise is averaged between two defined regions as shown in Figure 13.		
	Averaged region 1 to 1.5 minutes Averaged region 8.5 to 9 minutes		
	· · ·		
	A malanda		
	1 minute 1.5 minutes 8.5 minutes 9 minutes  Baseline Start (BL Start : Average Time) (BL end- Average Time) Baseline End		
	Figure 13 - Baseline Noise determination		

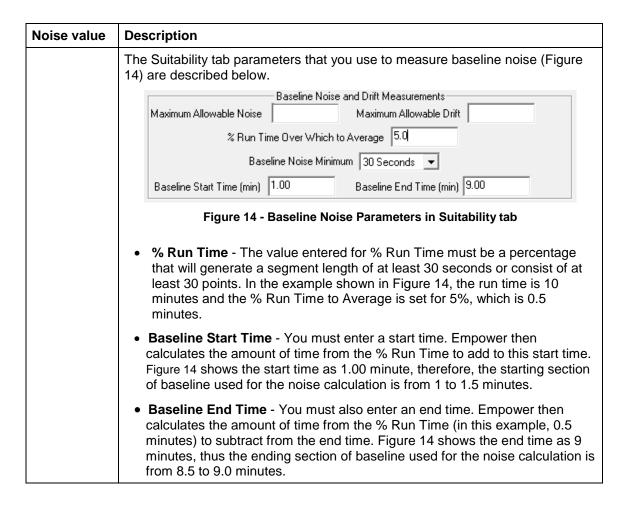


Figure 15 shows an example of how to configure the processing method Suitability tab parameters to calculate the s/n field using Baseline Noise as the Noise Value for s/n.

Figure 16 shows an example of the results obtained for s/n when using the Suitability tab parameters shown in Figure 15.

**NOTE:** Results are not shown for USP s/n, EP s/n, and JP s/n because the Calculate USP, EP, and JP s/n check box was not selected in the processing method Suitability tab in the Figure 15 example.

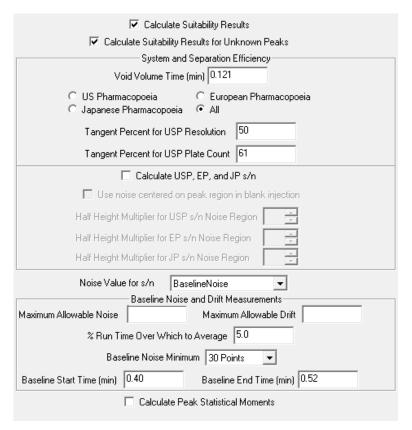


Figure 15 - Example Suitability tab parameters for calculating the s/n field using Baseline Noise

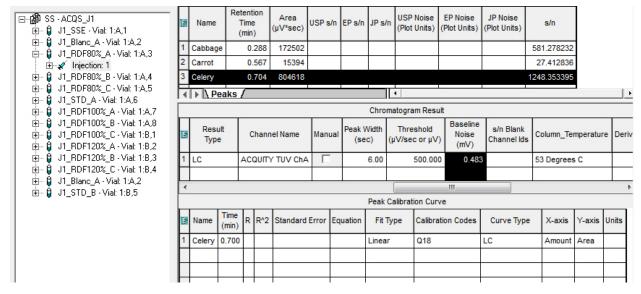


Figure 16 - Example results for s/n when using Baseline Noise as Noise Value for s/n

### Calculating the signal-to-noise ratio using a custom field

If none of Empower's formulas satisfy your requirements, you can create your own custom field. The following built in-fields in Empower can assist you in the creation of your own field:

- Peak to Peak Noise
- Average Peak to Peak Noise
- Detector Noise
- Average Detector Noise
- Baseline Noise
- Baseline Drift
- USP Noise
- EP Noise
- JP Noise
- Height
- Scale to µV

**Example 1: USP s/n** – The following example custom field replicates the calculated USP s/n (see Figure 17):

SIGNAL\_TO\_NOISE\_USP = 2 x (Height x Scale to  $\mu$ V – 0.5 x USP Noise) / USP Noise

This formula provides the same result as if you had selected to calculate USP s/n using a blank injection.

To simulate USP s/n as a built-in field as a confirmation calculation or to have any other calculation where you would like to calculate Peak to Peak Noise from a blank injection, proceed as follows:

- 1. In the Suitability tab of the processing method Select Calculate USP s/n, EP s/n and JP s/n.
- 2. Select Use noise centered on peak region on blank injection.
- 3. In the Alter Sample table, select the **Blank** check box next to the injection to be used as a blank.

You can use these same three steps to calculate USP Noise, EP Noise, or JP Noise accordingly for any custom field.

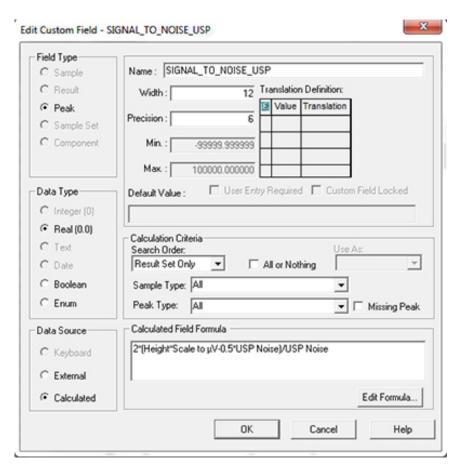


Figure 17 – Example custom field used to replicate USP s/n when using a blank injection

**Example 2: non-USP s/n** – The following example custom field does not incorporate the -1/2 USP noise factor (see Figure 18):

SIGNAL\_TO\_NOISE\_NON\_USP = 2 x (Height x Scale to  $\mu V$  / USP Noise

This custom field will only produce a result if USP Noise has a value. As in Example 1, you must do the following:

- 1. In the Suitability tab of the processing method Select Calculate USP s/n, EP s/n and JP s/n.
- 2. Select Use noise centered on peak region on blank injection.
- 3. In the Alter Sample table, select the **Blank** check box next to the injection to be used as a blank.

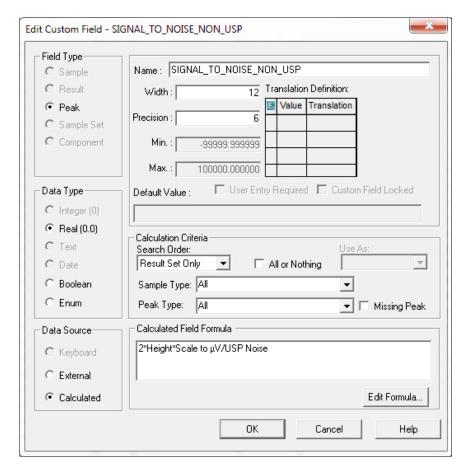


Figure 18 – Example custom field that does not incorporate the  $^{1\!\!/_{\! 2}}$  Peak to Peak Noise factor

**Example results** – The set of results shown in Figure 18 includes both the SIGNAL\_TO\_NOISE\_USP and SIGNAL\_TO\_NOISE\_NON\_USP custom fields as compared to the Empower built-in fields.

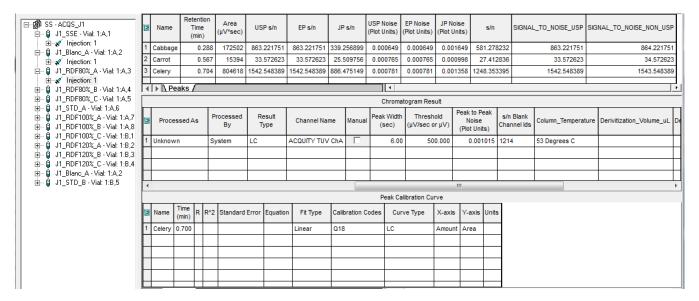


Figure 19 - Results for custom fields SIGNAL\_TO\_NOISE\_USP and SIGNAL\_TO\_NOISE\_NON\_USP