USP Signal-to-Noise in Empower 2

This Technical Note explains Waters' interpretation of the new USP signal-to-noise (S/N) calculation and presents a choice of implementations using custom field calculations. It contains the following topics:

- Discussion of USP Signal-to-Noise Definition
- Using a Custom Field to determine USP Signal-to-Noise by Making a Blank Injection (the preferred approach)
- Using a Custom Field to determine USP Signal-to-Noise Within one Chromatogram
- Differences between USP Signal-to-Noise and European Pharmacopeia Signal-to-Noise
- Generic Signal-to-Noise Determination in Empower 2

Discussion of USP Signal-to-Noise Definition

Until recently, the USP had not formalized their definition of Signal-to-Noise (S/N). Effective December 2009, the USP 32 standard defines it as follows:

> S/N = 2h/hn in which h is the height of the peak corresponding to the component concerned; and hn is the difference between the largest and smallest noise values observed over a distance equal to at least five times the width at the half-height of the peak and, if possible, situated equally around the peak of interest.

Given this simplistic definition of S/N, and with no clarifying figure or examples provided by USP, the correct approach to measuring noise is a key discussion point. The USP definition states that noise is "the difference between the largest and smallest noise values…" While this measurement as described is simple, it does not compensate for local systematic drift. It is assumed that the noise measurement should account for drift. Using this approach, noise will be overestimated when drift is present. As the noise increases, the S/N decreases. If S/N is subsequently used in the determination of the Limit of Detection (LOD)/Limit of Quantitation (LOQ), the LOD/LOQ values obtained will be larger than they would have been otherwise, making the assay appear less sensitive than it may actually be. It is therefore important to use the most representative noise value possible.

Waters' recommendation is to measure noise over the appropriate time region using the Peak-to-Peak Noise determination in Empower 2 software. The Peak-to-Peak Noise approach determines a best-fit regression line to the noise and calculates the residual amount for each data point. The Peak-to-Peak Noise calculation is the difference between the maximum residual from the best-fit line minus the minimum residual from the best-fit line. This approach corresponds to a visual inspection of noise, which is the intent of the USP noise description. Using the Peak-to-Peak noise value also has the benefit that the fitted regression line reduces the impact of drift on the noise calculation. Additionally, this approach corresponds to the ASTM definition of chromatographic noise.

The determination of Peak-to-Peak noise in Empower 2 is enabled in the Noise and Drift tab of the processing method (Figure 1). The USP S/N definition states that the noise interval (the time between the Start and Stop Time parameters) should be “equal to at least five times the width at the half-height of the peak” [of interest]. Empower 2 software also requires that the noise interval contains a minimum of 60 points in order for noise to be calculated. Hence, the data rate used during data acquisition must be set appropriately. The Segment Width parameter is used in the determination of Average Detector Noise and Average Peak-to-Peak Noise and is not relevant to the Peak-to-Peak noise determination.

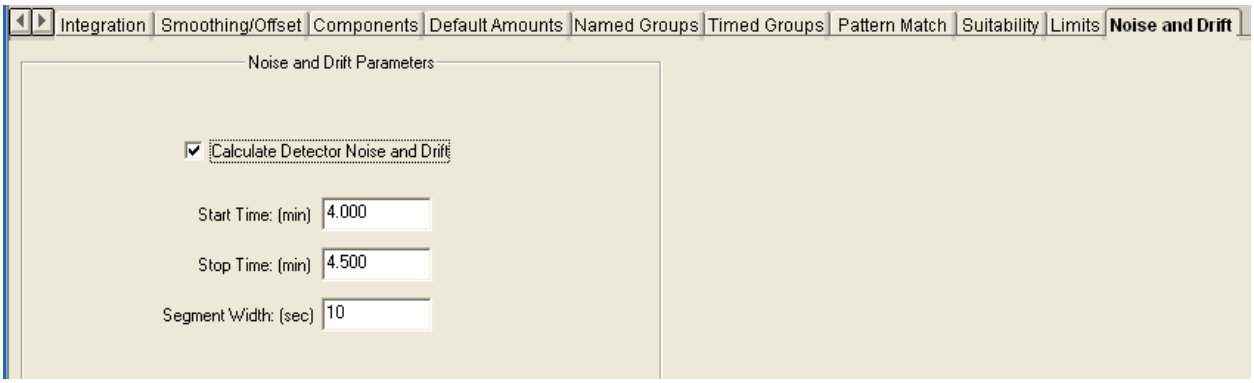

Figure 1 – Empower 2 Processing Method Window, Noise and Drift tab

The USP S/N definition states the time range used for the determination of noise should be “if possible, situated equally around the peak of interest”. Given this, and the fact that noise must be measured within a peak-free region of the chromatogram, it is preferable to use a blank injection for the determination of noise. However, it is simpler and fully acceptable to measure noise within the chromatogram of interest.

Using a Custom Field to Determine USP Signal-to-Noise by Making a Blank Injection

In Empower 2, the USP S/N calculation is best determined through the use of a custom field. The equivalent to the USP S/N formula of 2h/hn is as follows in Empower 2:

*USP S/N = 2*Height*Scale to µV/(Blank.1.SAME(Peak to Peak Noise))*

Figure 2 shows this formula and the appropriate custom field parameters in the Empower 2 Edit Custom Field window.

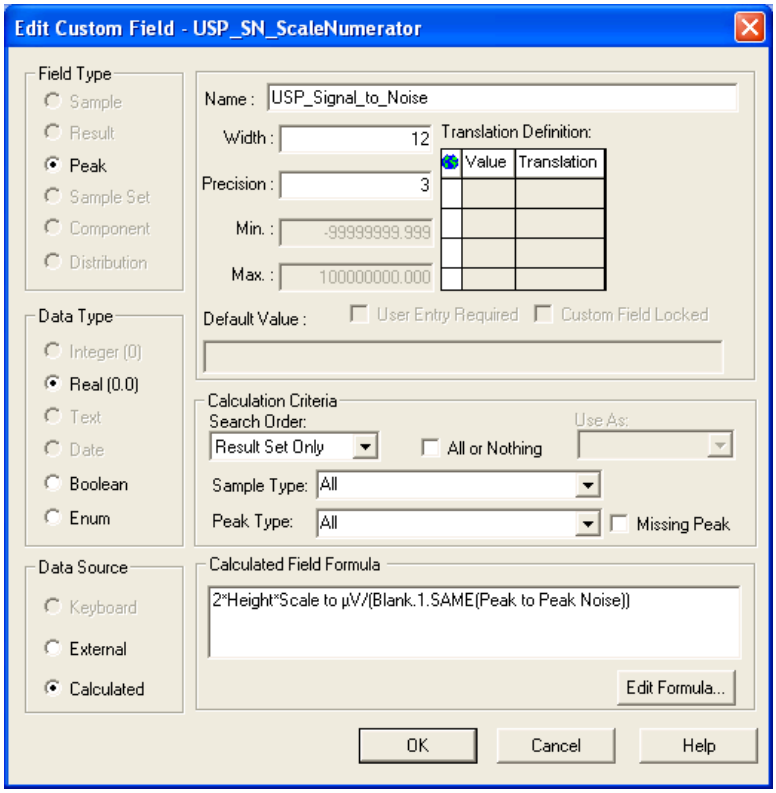

Figure 2 – Empower 2 USP Signal-to-Noise Custom Field, Making a Blank Injection

Note the following:

- The period-delimited syntax Blank.1.SAME represents the intersample calculation syntax and refers to the Label, Injection #, and Channel Name of the blank injection to be used for the noise determination. This example assumes that the blank injection has the string Blank specified in the Label field of the sample set method. The key word SAME in the Channel Name position of the syntax indicates that the channel (i.e., the detector signal) used for the blank injection will be the same as that for the injection of interest. Note that his detail is only relevant when using multiple detectors or acquiring other signals. This syntax can be modified as necessary for individual use.
- The Scale to µV field is the value used to scale the Height value to the same units as the noise value. This is necessary because different detectors provide signals in different voltage units. The Scale to µV field is automatically populated with the appropriate conversion factor. This allows for automatic scaling conversion using data from any detector without any modification to the custom field's formula.
- The Calculation Criteria of Result Set Only indicates that any necessary intersample injections (i.e., the blank injection) will be found in the same Result Set as the injections of interest. This means that the blank injection must be acquired within each sample set in which you are determining S/N. If you want to reference a blank injection that is not within the same sample set as the injections of interest, you can change this setting to Result Set First. Either way, the most-recently processed blank injection (blank result that has the highest Result ID and matches the appropriate Label syntax) will be used as the reference for determining the noise value.

Using a Custom Field to determine USP Signal-to-Noise Within one Chromatogram

If you want to simplify your approach and not refer to a blank injection, the required custom field formula is:

*USP S/N = 2*Height*Scale to µV/Peak to Peak Noise*

When using this approach, the intersample syntax and Search Criteria parameter are not relevant and do not need to be considered. However, it is necessary to use a noise region (Figure 1) that corresponds to a peak-free region of your chromatogram.

Figure 3 shows this formula and the appropriate custom field parameters in the Empower 2 Edit Custom Field window.

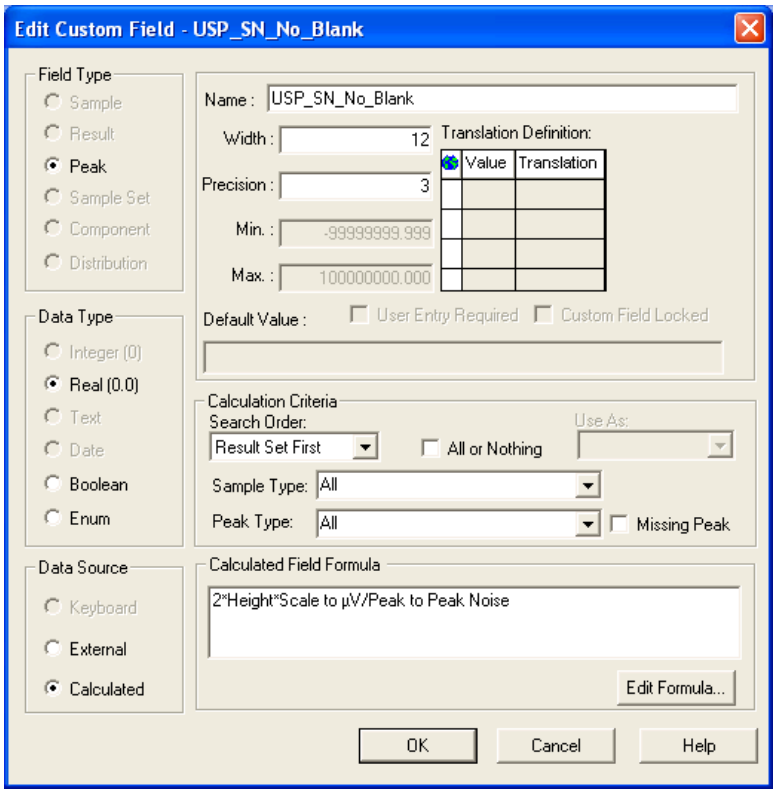

Figure 3 – Empower 2 USP Signal-to-Noise Custom Field, Determined within one Chromatogram

Differences between USP Signal-to-Noise and European Pharmacopeia Signal-to-Noise

In Empower 2, you can also determine European Pharmacopeia Signal-to-Noise (EP S/N).

The following is the definition of Signal-to-Noise ratio as defined in the 2005 European Pharmacopoeia (EP):

> Signal-to-Noise ratio – The Signal-to-Noise ratio (S/N) influences the precision of quantification and is calculated from the equation:
>
> $$S/N = \frac{2H}{\lambda}$$
>
> where:
>
> H = Height of the peak (Figure 4) 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 20 times the width at half-height.
>
> λ = Range of the background noise in a chromatogram obtained after injection or application of a blank, observed over a distance equal to 20 times the width at 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.

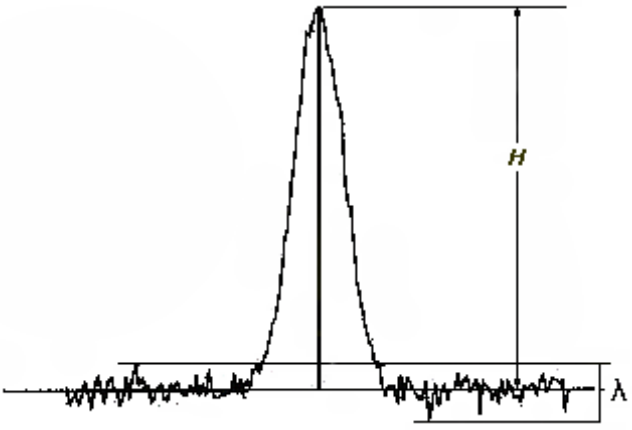

Figure 4 –EP Signal-to-Noise Determination

The Empower 2 Height calculation is slightly different than that defined in the EP. Empower 2 assumes that the peak has been integrated so as to draw the baseline at the bottom of the baseline noise. The peak height is then measured from the drawn baseline to the peak apex. EP defines peak height as the distance from the “maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half-height.”

This difference is corrected for in the Empower 2 calculation by subtracting ½ Peak-to-Peak Noise from the Height value. Therefore, the formula the Empower 2 uses for EP S/N is as follows (scaled appropriately for the detector units used):

EP S/N = 2 x (H – ½ Peak-to-Peak Noise) / Peak-to-Peak Noise

Although the intent of the new USP S/N definition is to harmonize the United States' and European definitions, there remain two primary differences between them:

1. The noise region for the EP S/N is specified as 20 times the width at half height and that of the USP S/N is specified as at least 5 times the width at half height.

2. The peak height calculation for the EP S/N specifies the measurement of peak height from the middle of the corresponding noise envelope (Figure 4) whereas the USP S/N definition does not imply any specific criteria.

The second difference causes the USP S/N calculation to consistently be higher than the EP S/N b y a value of 1.

Basic Formula	USP	EP
$S/N = 2h/h_n$	$2h_E/h_n$	$2(h_E - 0.5h_n)/h_n$
		$= 2h_E/h_n - h_n/h_n$
		$= 2h_E/h_n – 1$
		= USP - 1

where:

h_E = Peak height determined by Empower 2 software

h_n = Peak-to-Peak Noise

EP S/N is calculated when you select the Calculate EP s/n parameter in the Suitability tab of the processing method (Figure 5) and also specify the appropriate Detector Noise and Drift parameters in the Noise and Drift tab of the processing method (Figure 1). Like the USP S/N, EP S/N uses Peak-to-Peak Noise as the noise value in this determination. For additional information on EP S/N, refer to Technical Note TECN1852625, Understanding the EP Signal to Noise Calculation in Empower 2.

Generic Signal-to-Noise Determination in Empower 2

Signal-to-Noise is calculated when you specify a noise value within the Noise Value for s/n parameter in the Suitability tab of the processing method (Figure 5) and also specify the appropriate Detector Noise and Drift parameters in the Noise and Drift tab of the processing method (Figure 1). This determination is independent from the determination of EP S/N. Signal-to-Noise is determined using the Peak Height divided by the noise specified as the Noise Value for S/N.

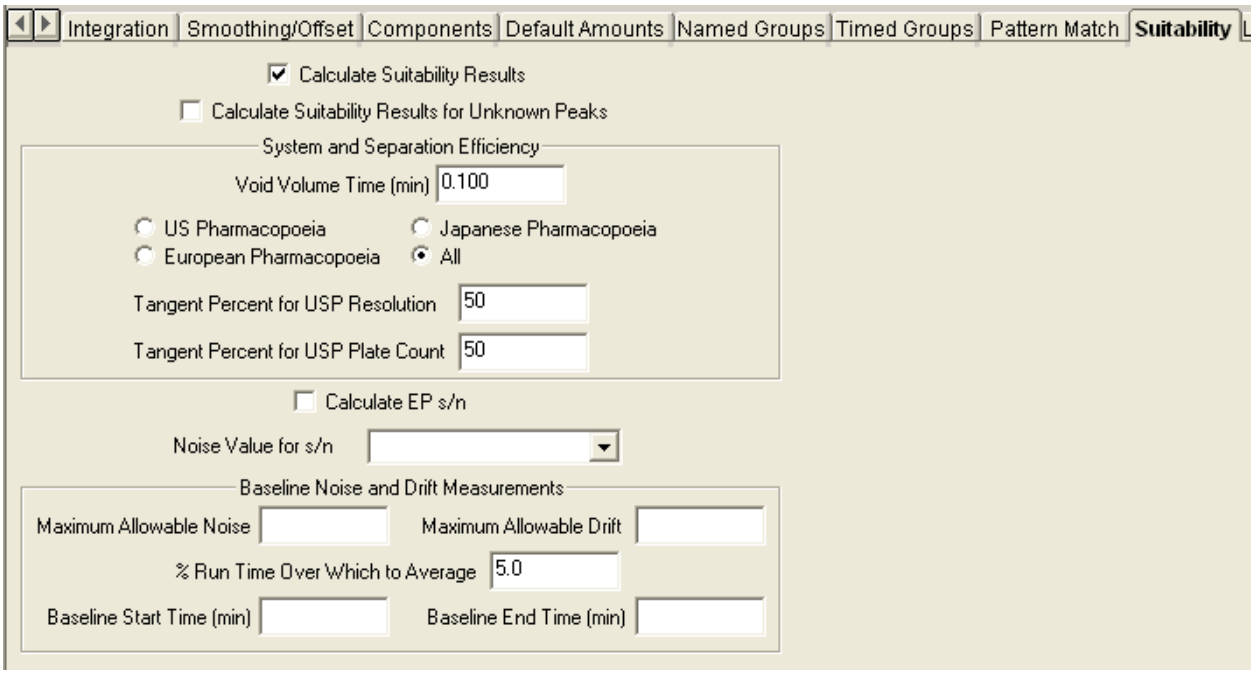

Figure 1 – Empower 2 Processing Method Window, Suitability Tab

Reference Information

- ASTM Designation E1657-98 Standard Practice for Testing Variable-Wavelength Photometric Detectors Used in Chromatography
- Waters Technical Note TECN1852433, Detector Noise and Drift Calculations
- Waters Technical Note TECN1852625, Understanding the EP Signal to Noise Calculation in Empower 2

NOTE: *When making any changes to a system, you should consider the applicable Standard Operating Procedures (SOPs) and complete the appropriate documentation and validation.*