Understanding the EP Signal-to-Noise Calculation in Empower 2

This document:

- Provides a definition of the 2005 European Pharmacopeia (EP) signal-to-noise ratio
- Describes the built-in EP S/N calculation in Empower™ 2
- Describes the custom field necessary to determine EP S/N using noise from a blank injection.

2005 European Pharmacopeia (EP) Definition: Signal-to-Noise Ratio

The signal-to-noise ratio (S/N) influences the precision of quantification and is calculated by the equation:

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

where:

H = Height of the peak (Figure 1) 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.

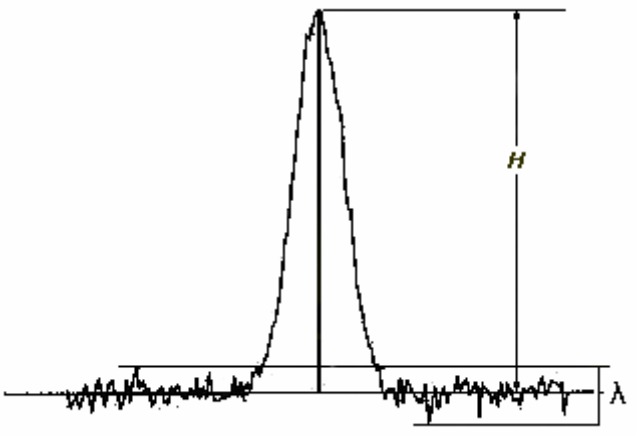


Figure 1 – Peak Height Measurement

In Empower 2 software, the EP Signal-to-Noise (EP S/N) is determined in an automated fashion and does not require a blank injection. The intention of this calculation is to preserve the sense of the EP calculation without requiring a separate blank injection. The use of a blank injection does not allow for this calculation to be determined automatically. If you need to calculate EP Signal-to-Noise using noise from a blank injection, you can do so by using a custom field.

EP Signal-to-Noise Calculation in Empower 2

The EP S/N calculation in Empower 2 is as close as possible to the EP definition, while not requiring a separate blank injection. The formula is as follows:

EP S/N = 2 x (H – ½ PPNoise / Scaling) / (PPNoise / Scaling)

where:

H	=	The absolute value of the height of the peak
PPNoise	=	The result's peak-to-peak noise value
Scaling	=	The scale to µV value

The Scale to µV field is the value used to scale the plot units to µV when calculating peak heights, areas, and S/N values. This field is also used to scale the noise field to µV units.

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.

Empower 2 peak-to-peak noise is the difference between the maximum residual from the best fit to the noise line minus the minimum residual from the best fit to the noise line (Figure 2).

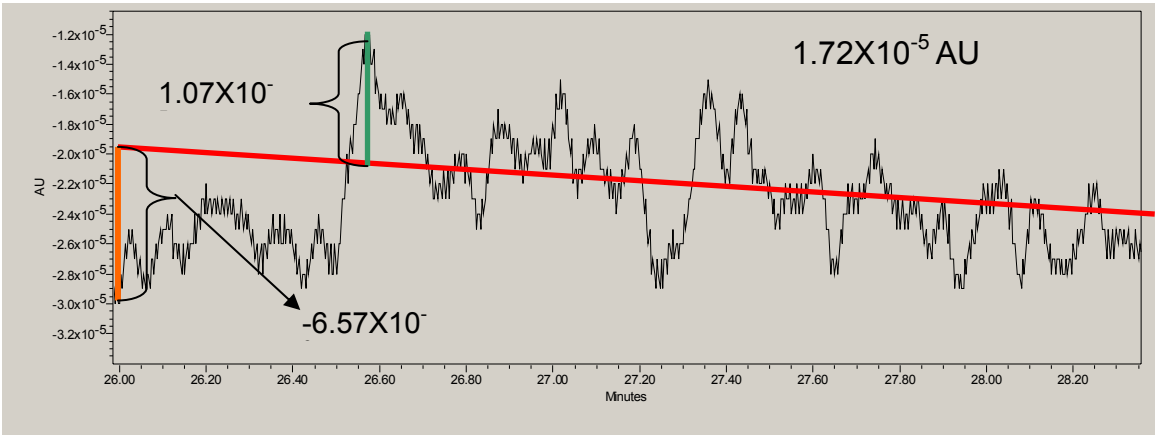


Figure 2 – Detector Noise = (Max(yi - ypi))- (Min(yi - ypi))

For clarity, the Empower 2 and EP calculations and the corresponding variables are summarized in Table 1.

Table 1 – Empower 2 and EP S/N Calculations and Corresponding Variables

Field	Empower 2	Empower 2 Definition	European Pharmacopeia	EP Definition
S/N	2 x (H – ½ PPNoise / Scaling) / (PPNoise / Scaling)		2H / λ	
S	H – ½ PPNoise / Scaling	Height measurement measured from peak maxima to bottom of noise, adjusted by ½ peak-to-peak noise in order to comply with the EP definition. Also adjusted by a scaling factor appropriate for the appropriate detector units.	H	Measurement from peak maxima to midpoint of baseline noise
N	PPNoise / Scaling	Range of background noise (maximum noise minus minimum noise) in the chromatographic region defined in the Noise and Drift tab in the processing method, adjusted by a scaling factor appropriate for the appropriate detector units.	N	Range of background noise (maximum noise minus minimum noise) in the chromatographic region of the component of interest +/- 10 times PW at ½ height

In order to calculate peak-to-peak noise appropriately, Waters recommends that you use a Start and End time that corresponds to a peak free region in the chromatogram equal to 20 times the peak width at ½ height for the component of interest.

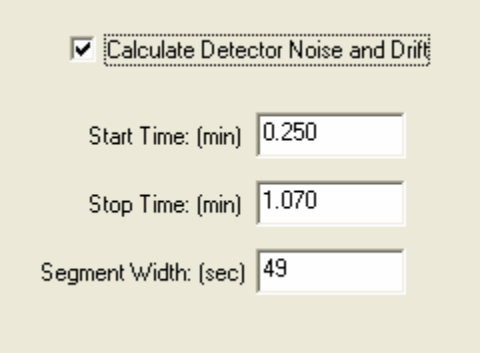


Figure 3 – Settings on the Noise and Drift Tab of the Processing Method

EP S/N Calculation in Empower 2 Using Blank Injection

NOTE: *You can find the custom field described here in the Custom Fields Default project located on your Empower 2 DVD media.*

It is possible to calculate EP S/N using the noise value from a blank injection by using a custom field. The formula, which is a rearrangement of the formula described in EP Signal-to-Noise Calculation in Empower 2 on page 2.is as follows:

(2 x Height/(Blank.1.SAME(Peak-to-Peak Noise)/Blank.1.SAME(Scale to µV)))-1

Blank.1.SAME represents intersample syntax in the format of Label.injection.channel. This formula assumes that the blank injection includes a label of *Blank*. The first injection of the blank is used. You can modify this syntax to suit your needs. This custom field is shown in Figure 4.

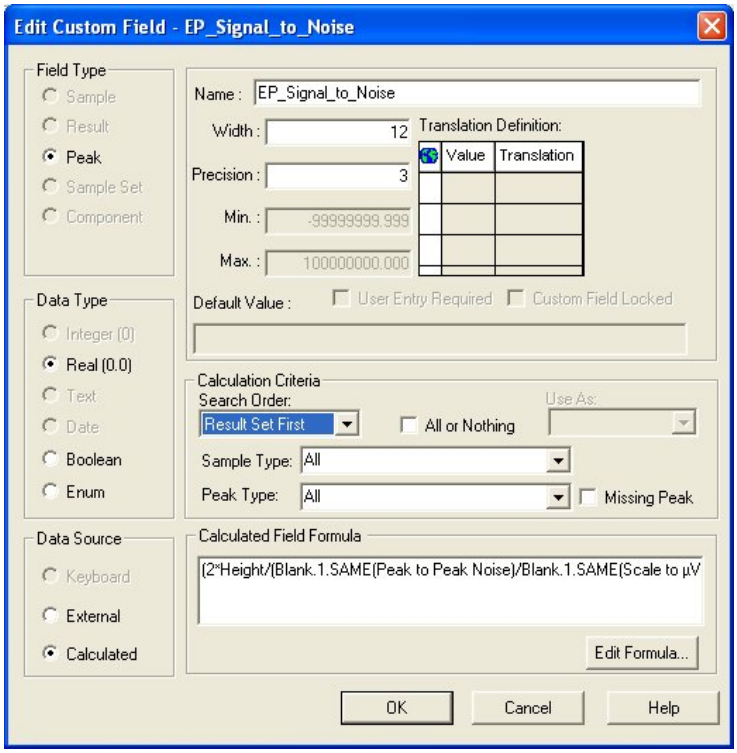
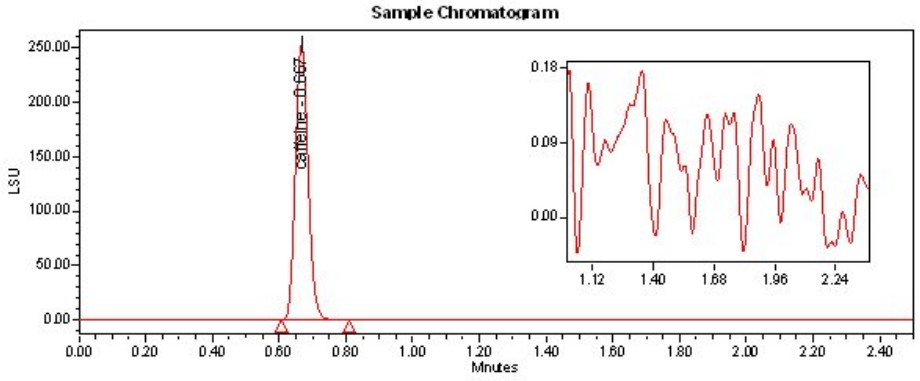
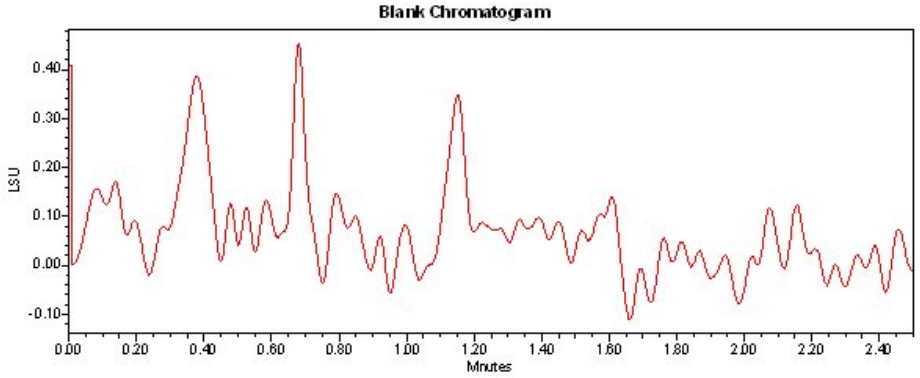


Figure 4 – Overview of EP Signal-to-Noise Calculation

Note that this calculation is based on two injections: the first is a sample and the second is a blank. This is ELSD data and the detector reports in Light Scattering Units. The numerical values were obtained from System Suitability, Noise and Drift, and Traditional Integration processing.

The results are shown in Figure 5.





EP S/N using Blank Injection
(ELSD Data)

	Name	RT	EP SN Blank Inj 1	PPNoise from Blank	Width @ 50 %	Height
1	caffeine	0.667	942.248587	0.537319	4.134318e-002	253413

Figure 5 – Data Used in Calculating EP S/N Using a Blank Injection

Conclusion

In Empower 2 software, the EP Signal-to-Noise (EP S/N) is determined in an automated fashion and does not require a blank injection. The intention of this calculation is to preserve the meaning of the EP calculation as closely as possible while still allowing it to be determined in an automated fashion. The use of a blank injection does not allow for this calculation to be determined automatically. If you need to calculate EP Signal-to-Noise using noise from a blank injection, you can do so by using a custom field as described in this document. Since each method of determining EP Signal-to-Noise uses a different noise determination, the resulting values will be different. It is up to the you to determine which calculation satisfies your corporate requirements.

NOTE: *When making any changes to a system, you should consider the applicable Standard Operating Procedures (SOPs) and complete the appropriate documentation and validation. If you have questions or require additional information, contact your local Waters Data Specialist.*