



## USP Chapter <621> Frequently Asked Questions: Calculating Signal-to-Noise

United States Pharmacopeia (USP) Chapter <621> on chromatography was recently updated to harmonize with the European Pharmacopoeia (EP). With this harmonization there are changes to how some of the system suitability calculations are done in Waters™ Empower™ Software, including:

- **Resolution** now uses peak width at half height
- **Plate Count** is now called **Plate Number**, and also uses peak width at half-height
- **Signal-to-noise** ratio now requires a blank injection from which noise is calculated at the expected retention time of a peak

We have received many questions from customers about calculating signal-to-noise ratio and what follows are responses to some of the most frequently asked questions.

**Q: Is Empower Software currently able to calculate signal-to-noise (s/n) according to USP/EP/JP following the changes to USP Chapter <621>?**

A: Yes, Empower Software can calculate signal-to-noise per the requirements of the various pharmacopeia. The changes to USP Chapter <621> were done to harmonize with the EP. The noise should be calculated at the expected retention time from a blank injection. There is a window around that expected retention time which is determined by the peak width at half height times five.

**Q: What is the recommended half-height multiplier, five or 20?**

A: The half-height multiplier for both USP and EP is five. This is the default value in most versions of Empower Software. This parameter can be easily adjusted if the default value is something other than five.

**Q: How do we identify which blank to use and how would we use multiple blanks in the Sample Set?**

A: A blank injection should occur before the samples of interest. There is a field called Blank (which is a hidden column by default) in the Sample Set Method with a check box. Check the box for the blank or blanks you would like to use for the noise calculation. If you select multiple blanks, or if you make multiple injections of one blank, Empower Software will calculate an average noise, which will be used in the signal-to-noise calculation.

**Q: What do you recommend if there is interference in the blank at the expected retention time of my peak?**

A: You can use the Noise and Drift tab to select a desired section of the blank injection for the calculation of noise. Create an inter-sample custom field to determine signal-to-noise in the samples. For example, if you had one injection of a blank and the label was "Blk", the custom field would be:

$$2*(Height-(0.5*Blk.1.(Peak\ to\ Peak\ Noise/Scale\ to\ \mu V)))/Blk.1.(Peak\ to\ Peak\ Noise/Scale\ to\ \mu V)$$

To specify the region in the blank, select peak-to-peak noise from the Noise Value for s/n list and specify the region in the Noise and Drift tab.

**Q: Will a steep baseline, due to the gradient, affect the signal-to-noise calculation?**

A: Yes, the calculated noise will be higher on the steep portion of the baseline as compared to a flat portion of the baseline. This will result in a lower signal-to-noise value. If the baseline is reproducible, then you will have consistent results for signal-to-noise ratio. If the baseline is not reproducible, then it is recommended that you select a different section of the baseline in the blank and use the custom field described in response to question number four.

**Q: Can you use the signal-to-noise calculation to determine Limit of Detection and Limit of Quantitation?**

A: Yes, Limit of Detection is classically defined as a signal-to-noise ratio of 3:1 and Limit of Quantitation as 10:1.

**Q: When would you use the Noise and Drift tab to calculate noise?**

A: The Noise and Drift tab is used when determining noise within the same chromatogram with the peaks of interest. It is also used if you want to create an inter-sample custom field to determine noise within a specified region of the blank injection.

**Q: When would you select the noise from the Noise Value for s/n drop down menu?**

A: If you want to calculate signal-to-noise for non-pharmacopoeia methods, you can select the type of noise to be used in the calculation.

**Q: What is Baseline Noise and when would it be used?**

A: Empower Software calculates Baseline Noise based on the maximum change in signal over a selected time period. The reported baseline noise value is an average of several intervals as determined by the % Run Time Over Which to Average field. Baseline Noise could be used for the noise in signal-to-noise calculations for non-pharmacopoeia applications (reference question eight).

**Q: Would it be feasible to calculate an average signal-to-noise ratio from various blank runs and incorporate a standard deviation as to what is an acceptable signal-to-noise ratio that can differentiate noise from a peak of interest?**

A: Standard deviation, or sigma, indicates the spread of values around the mean value. A Summary Report can easily be generated that automatically calculates standard deviation for any numerical field, including USP s/n. Alternatively, an inter-sample custom field could be created to calculate the standard deviation where you back calculate from the average and %RSD custom fields. When calculating with custom fields, the reported values are permanently stored in the database.



Waters, and Empower are trademarks of Waters Corporation.  
All other trademarks are the property of their respective owners.