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# Understanding Spectral Bandwidth and Resolution in the Regulated Laboratory

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Key Words

- British Pharmacopeia (BP)
- European Pharmacopeia (EP)
- Regulatory Compliance
- Resolution
- Spectral Bandwidth
- Toluene in Hexane Solution
- U.S. Pharmacopeia (USP)
- UV-Visible Spectrophotometer

## Introduction

Selecting the appropriate spectrophotometer to comply with the complex requirements of a regulated laboratory can be a daunting task.[1] Two specifications that have become increasingly challenging are resolution and spectral bandwidth. Confusion over the requirements for regulatory compliance has led, in particular, to two widespread myths regarding spectral bandwidth and resolution.

Myth 1: *Photometric analysis requires a spectrophotometer with a 1.0 nm spectral bandwidth for regulatory compliance.*

Myth 2: *A smaller spectral bandwidth generates higher quality data.*

This application note will address these two myths by explaining the principles of spectral bandwidth and resolution. Furthermore, it will address the regulatory requirements for these specifications in the pharmaceutical industry as dictated by the pharmacopeias of the United States, Europe and Britain referred to in this document as the USP, EP and BP, respectively.

## What is Spectral Bandwidth?

When a monochromator is set to a particular wavelength, light with a Gaussian intensity distribution of wavelengths emerges from the exit slit. This intensity distribution is depicted in Figure 1. In this figure, $\lambda_1$ and the dotted vertical line represent the wavelength set on the instrument. The heavy black line represents the distribution of light that reaches the sample. The spectral bandwidth is defined as the width of the band of light at one-half the peak maximum (or full width at half maximum [FWHM]) and is represented by the two vertical red lines and $\lambda_{SB}$ on the wavelength axis. Spectral slit width, a related term frequently referenced in EP regulations, is defined as the total spread of wavelengths represented by the blue lines and $\lambda_{SSW}$ in Figure 1. The spectral bandwidth of the instrument will always be narrower than the spectral slit width.

The spectral bandwidth of a spectrophotometer is related to the physical slit-width of the monochromator and, therefore, to the resolution capabilities of the instrument. To the best of our knowledge, there are no formal specifications for spectral bandwidth defined in any Pharmacopeia. Some pharmacopeias do call out the advantages of a variable bandwidth instrument. Assay optimization on a variable bandwidth instrument will be discussed in the following sections.

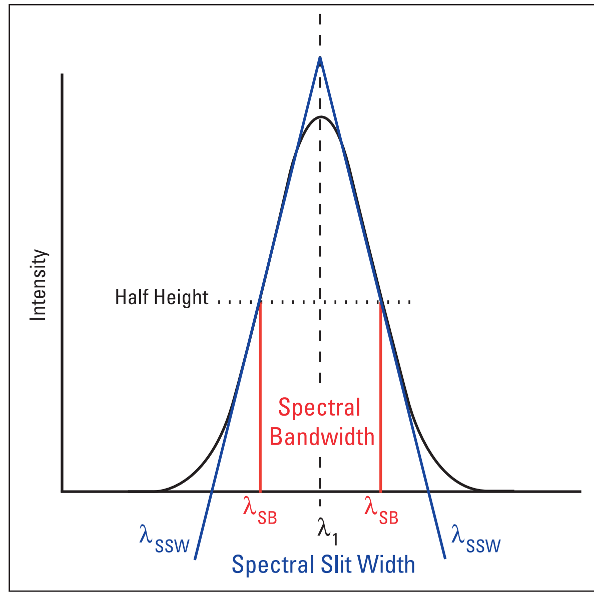


Figure 1: Gaussian intensity distribution of wavelengths emerging from the monochromator. The spectral bandwidth is defined by the red boundaries and $\lambda_{SB}$. The spectral slit width is depicted by the blue boundaries and $\lambda_{SSW}$.

## What is Resolution?

Reduced to its most basic definition, resolution is defined as the ability of an instrument to separate light into finite, distinct wavelength regions and distinguish these finite regions from each other. Resolution is primarily governed by the physical slit width of the instrument, in combination with the inherent optical dispersion of light from the exit of the monochromator to the detector of the instrument. Reducing the physical slit width decreases the spectral bandwidth and improves the ability of the instrument to resolve closely spaced peaks.

In the pharmaceutical industry, a 0.02% solution of Toluene in Hexane is the currently accepted standard for the verification of the resolution of a spectrophotometer. The resolving capacity of the spectrophotometer is calculated using the ratio between the absorbance maximum and minimum of the standard. Table 1 depicts the correlation of spectral bandwidth to resolution for a 0.02% solution of toluene in hexane as the ratio between the maximum and minimum absorbance of the solution.

| Spectral Bandwidth | 3.0 nm ± 0.2 nm | 2.0 nm ± 0.2 nm | 1.5 nm ± 0.1 nm | 1.0 nm ± 0.1 nm | 0.5 nm ± 0.1 nm |
|---|---|---|---|---|---|
| Resolution | 1.0 – 1.1 | 1.3 – 1.4 | 1.6 – 1.7 | 1.9 – 2.0 | 2.3 – 2.4 |

Table 1: The correlation of spectral bandwidth to resolution for a Toluene in Hexane Solution Standard measured at ambient temperature (25 °C)

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## What are the Regulatory Requirements for Resolution?

- **USP:** No specific requirement is given in Section 851 of the USP, which provides the specifications for UV-Visible spectrophotometers.
- **EP** (or Ph. Eur.): The current version of the EP does not contain any general instrument requirements for spectral bandwidth. It does include requirements for testing the resolution of the spectrophotometer and choosing the slit-width when a variable slit-width spectrophotometer is used. In method 2.2.25, the EP describes these requirements as follows:
  - **For Qualitative Analysis:** "When prescribed in a monograph, measure the resolution of the apparatus as follows: record the spectrum of a 0.02 per cent *V/V* solution of *toluene R* in *hexane R*. The minimum ratio of the absorbance at the maximum at 269 nm to that at the minimum at 266 nm is stated in the monograph."
  - **For Quantitative Analysis:** "To avoid errors due to spectral slit-width, when using an instrument on which the slit-width is variable at the selected wavelength, the slit-width must be small compared with the half-width of the absorption band but it must be as large as possible to obtain a high value of $I_0$. Therefore, a slit-width is chosen such that further reduction does not result in a change in absorbance reading."

  ***To the best of our knowledge the only monograph that currently specifies a required resolution for the spectrophotometer is monograph 0113 for Benzylpenicillin potassium. This monograph specifies a resolution of at least 1.7.***
- **BP:** The 2008 edition of the British Pharmacopeia refers to method 2.2.25 of the EP for instrument requirements. Within the BP, specific monographs may require an instrument with a bandwidth < 2.0 nm.

## Advantages of a Variable Bandwidth Spectrophotometer

For laboratories which require compliance to multiple regulatory agencies for a variety of applications, the variable bandwidth spectrophotometer offers a simple solution. It is important when using a variable bandwidth instrument to recognize that it is not ideal to use the narrowest available bandwidth at all times. Reducing the bandwidth leads to a reduction in energy reaching the sample. This may result in the reduction of:

- Data Precision and Repeatability
- Accuracy of Results
- Detection and Quantitation Limits
- Method Robustness

When performing quantitative measurements it is important to maximize the signal-to-noise ratio for the compound(s) of interest. This is achieved by optimizing the ratio of the peak absorbance measured with respect to the noise associated with the baseline (signal-to-noise ratio). The spectral bandwidth selected for an analysis should be close to, but not less than, 1/10 of the width of the absorbance peak at half-height. **A variable bandwidth instrument allows the analyst to balance the need to resolve spectral features with the ability to achieve the desired limits of detection and quantitation.**

In the following illustrations, different compounds were analyzed at bandwidths of 0.5, 1.0, 1.5, 2.0 and 4.0 nm. The signal-to-noise ratios of the selected peaks were calculated and the combined information was plotted. As you can see from Figure 2, the signal-to-noise ratio of Compound A is maximized at 1.0 nm; however, the signal-to-noise ratio of Compound B is maximized at 2.0 nm. In this case, we can conclude that the analyses of these compounds can be optimized at different bandwidths, especially when low limits of quantitation and detection are desired.

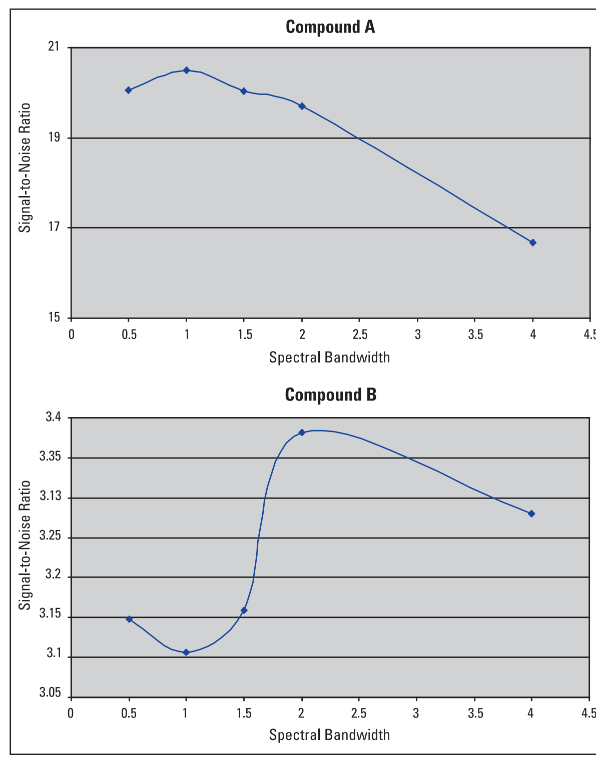


Figure 2: Graphical display of signal-to-noise ratio vs. spectral bandwidth for Compounds A and B. The maximum of the plot for Compound A indicates the maximum SNR occurs at a spectral bandwidth of 1.0 nm. The maximum of the plot for Compound B indicates the maximum SNR occurs at a spectral bandwidth of 2.0 nm.

## Conclusion

Many 1.0 nm fixed bandwidth instruments are available; however, the optimal bandwidth for an analysis is not necessarily 1.0 nm. Although a narrower spectral bandwidth does improve the resolution of closely spaced peaks, it also decreases the signal-to-noise ratio. Consequently, a spectral bandwidth should be chosen based on both the needs of the analysis being performed and within the regulatory guidelines of the appropriate pharmacopeia, when applicable.

The Thermo Scientific Evolution 300 and Evolution 600 variable bandwidth UV-Visible spectrophotometers are designed to accommodate the needs of a variety of applications. These instruments have the flexibility to adjust the bandwidth of the instrument for measurements at bandwidths of 0.2, 0.5, 1.0, 1.5, 2.0, and 4.0 nm.[2] A wide range of fixed spectral bandwidth instruments are also available for routine applications at 1.0, 1.8, 2.0 and 5.0 nm. An NIST-traceable Toluene in Hexane Solution Standard is offered for verifying the resolution of any spectrophotometer.

## References


1. For more information on these requirements, see Thermo Scientific Application Note AN51111 *U.S. and European Pharmacopeias and UV-Visible Spectrophotometers*.
2. A spectral bandwidth of 0.2 nm is only available with the Evolution™ 600 Spectrophotometer.


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