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應用手冊

Optimization of Detector Parameters to Improve Sensitivity using the Alliance™ iS HPLC System with PDA Detector

Lise Gauthier, Paula Hong

Waters Corporation, United States

Published on June 23, 2025

Contact Sales

Abstract

Default PDA instrument method settings are frequently used for routine HPLC analysis. While these default settings are often suitable, they may need to be modified to obtain optimal separations. In this study, the USP method for organic impurities in ibuprofen tablets was used to demonstrate how optimizing detector settings produced an improvement in sensitivity.

The Alliance iS HPLC System with PDA Detector is a newly designed PDA detector that has additional tools to improve performance, including variable slit width and resolution range to 20 nm. For this study, the impact of

the following detector settings on sensitivity (defined as the USP signal-to-noise (S/N) ratio) was evaluated: data rate, filter time constant, slit width, resolution, and absorbance compensation. Using optimized detector parameters produced a 7x increase in the USP S/N ratio over that obtained using the default values. The study shows how, with minimal effort, the detector parameters can be easily optimized resulting in an improvement in sensitivity.

Benefits

- The Alliance iS HPLC System with PDA Detector allows the user to easily modify PDA settings to obtain optimal performance, including signal-to-noise
- The Alliance iS HPLC System with PDA Detector has a range of parameters, including variable slit width and wide resolution range for method optimization
- Optimization of the PDA settings produced a S/N ratio increase of 7x

Introduction

Default PDA instrument method settings are frequently used for running HPLC methods. When running a method for the first time, these default settings are a good starting point and often produce suitable chromatography. However, detector parameters may need to be modified to obtain optimal separations. In this study, the USP method for organic impurities in ibuprofen tablets was used to show how the Alliance iS HPLC System with PDA Detector parameters may be optimized to improve sensitivity. The USP S/N ratio was used to define sensitivity. The method is a reversed-phase isocratic method with UV detection (254 nm). The method's system suitability criteria includes a S/N ratio of not less than (NLT) 10 for a 5-ppm solution of ibuprofen.

The Alliance iS HPLC System with PDA Detector settings that were evaluated and optimized for their impact on sensitivity included data rate, filter time constant, slit width, resolution, and absorbance compensation.

- Data rate: The data rate defines the rate that the detector collects data, measured in hertz (Hz). The data rate should be set to yield reproducible peak area and retention times. Data rates that are too low will result in poorly defined peaks; high data rates may result in increased noise. Generally, the data rate is set to collect 25-50 points across the narrowest peak in the chromatogram. The data rate of the Alliance iS HPLC System

with PDA Detector has settable values of 1–160 Hz. The default data rate is 10 Hz.

- Filter time constant: The filter time constant is a noise filter that filters out high frequency noise. Faster time constants produce narrower peaks and remove less baseline noise. Slower time constants result in broader peaks and decreased baseline noise. The Alliance iS HPLC System with PDA Detector has the following filter time constant options: no filter, slow, normal, fast, and custom. The default selection is normal.
- Slit width: Slit width is an added feature available in the Alliance iS HPLC System with PDA Detector. The slit width determines the amount of light that reaches the photodiode array sensor which impacts resolution. Smaller slit widths result in improved resolution. Larger slit widths produce less noise and increased sensitivity, but at the cost of lower resolution. The Alliance iS HPLC System with PDA Detector has a variable slit, with available slit width sizes of 35 µm, 50 µm, 100 µm, and 150 µm. The detector's default slit width is 50 µm.
- Resolution: The resolution setting determines bandwidth, which is the number of diode responses that are averaged when calculating the absorbance at a specified wavelength. Larger values result in less noise and a better S/N ratio, but they also produce decreased spectral resolution and can impact linearity. Resolution on the Alliance iS HPLC System with PDA Detector is settable over a range of 1–20 nm. The default value is 4 nm.
- Absorbance compensation: The absorbance compensation feature of the Alliance iS HPLC System with PDA Detector provides a way to reduce non-wavelength dependent noise. This is achieved by collecting absorbance data over a user-specified wavelength range where there is little or no absorption and calculating the average absorbance. This average absorbance is then subtracted from the absorbance value. The Alliance iS HPLC System with PDA Detector default setting for absorbance compensation is 'off'.

Using the USP method, each of these detector settings were individually evaluated and optimized. A method was created using the optimized settings and the results were compared to those obtained using default values.

Experimental

Method: USP ibuprofen tablets organic impurities

Sample Description

Sensitivity Solution Preparation

The sensitivity solution was prepared using ibuprofen purchased from MilliporeSigma. The ibuprofen was serially diluted with mobile phase to a final concentration of 0.005 mg/mL.

LC Conditions

LC system:	Alliance iS HPLC System with PDA Detector
Detection:	PDA Detector with 10 mm flow cell
Wavelength:	254 nm
Sampling rate:	Various
Vials:	LCGC Certified Clear Glass, 12 x 32 mm, Screw Neck Vial with cap and preslit PTFE/Silicone Septum, 2 mL Volume, 100/pk (p/n: 186000307C)
Column:	XBridge™ BEH™ C_{18}, 250 x 4.6 mm; 5 µm (p/n: 186003117)
Column temperature:	25.0 °C
Sample temperature:	15.0 °C
Injection volume:	10.0 µL
Flow rate:	2.0 mL/min
Mobile phase:	4g/L Chloroacetic Acid in 40:60 water:acetonitrile, pH 3.0
Gradient:	Isocratic

Needle wash:	90:10 methanol:water
Seal wash:	90:10 water:acetonitrile

Data Management

Chromatography data system:	Empower™ Chromatography Data System (CDS)

Results and Discussion

To assess the impact of PDA settings on detector noise and the method's signal-to-noise performance, a controlled study was performed. One variable was adjusted at a time, with all other settings being either the default or the optimized value determined from the previous set of experiments. The order of experiments was selected to assess those characteristics that are most commonly adjusted and/or have a larger impact on sensitivity.

The order of experiments was: data rate, filter time constant, slit width, resolution (2D), and absorbance compensation.

1. Data rate

The 5-ppm ibuprofen system suitability solution was analyzed with data rates of 1, 2, 10, and 40 Hz. The filter time constant, slit width, resolution, and absorbance compensation were kept at their default values. The results are shown in Table 1. Based on these results, the best data rate for the method is 2 Hz. At a data rate of 2 Hz, there were 31 points across the peak, which is within the desired range of 25–50 points. The USP S/N ratio was 25, which meets the defined acceptance criteria.

Data rate (Hz)	Noise (µAU)	Points across the peak	USP S/N
1	16	24	25
2	20	31	25
10 (Default)	70	105	7
40	174	346	3

Table 1. Results at various data rates.

2. Filter time constant

After the data rate was defined, the filter time constant was examined. With the data rate at 2 Hz, the system suitability solution was analyzed using slow, normal, fast, and no filter time constants. The slit width, resolution, and absorbance compensation were kept at their default values. The results are presented in Table 2. The system suitability criteria for S/N was not met when no filter was applied. The highest S/N ratio was obtained using a slow filter time constant.

Filter time constant	Noise (µAU)	USP S/N
Slow	12	33
Normal (default)	20	25
Fast	48	10
NO filter	64	6

Table 2. Noise and S:N results with different filter time constants.

3. Slit width

Slit width was examined to determine its impact on sensitivity. The 5-ppm ibuprofen system suitability solution was analyzed using slit widths of 35 µm, 50 µm, and 150 µm. The data rate was 2 Hz and the filter time constant, resolution, and absorbance compensation were kept at their default values. The results, shown in Table 4, show that the S/N ratio acceptance criteria of NLT 10 was met with each of the slit widths. Only small differences in the S/N ratio were seen across the different slit widths.

Slit width (µm)	Noise (µAU)	USP S/N
35	23	20
50 (Default)	20	25
150	17	28

Table 3. Slit width results.

4. Resolution

With the data rate set at 2 Hz, the 5-ppm ibuprofen system suitability solution was analyzed with resolution settings of 1, 4, 8, 12, 16, and 20 nm. Default settings were used for the filter time constant, slit width, and absorbance compensation. The results are shown in Table 3. Overall, the S/N ratio acceptance criteria of NLT 10 was met, and there was little variation in the results with the different resolution settings.

Resolution (nm)	Noise (µAU)	USP S/N
1	25	20
4 (default)	20	25
8	21	24
12	18	28
16	24	23
20	22	26

Table 4. Resolution results.

5. Absorbance compensation

The absorbance compensation feature was evaluated for its impact on the S/N ratio. Using the previously optimized detector parameters (a data rate of 2 Hz; a slow filter time constant; a slit width of 50 µm; and resolution at 4 nm), the system suitability solution was analyzed with and without absorbance compensation. The wavelength range used for compensation should be selected based on the spectral range of the analyte. For this analysis, the default compensation wavelength range of 310–410 nm, where there was no absorbance, was used for compensation. The impact of absorbance compensation is shown in Table 5. With absorbance compensation, a reduction in noise was observed and a 1.5x increase in the S/N ratio.

Data rate (Hz)	Filter time constant	Resolution (nm)	Compensation	Slit width (µm)	Noise (µAU)	USP S/N
2	Slow	4	Off	50	12	33
2	Slow	4	On (310–410 nm)	50	8	51

Table 5. Results with and without absorbance compensation.

Based on the set of experiments outlined, data rate, filter time constant, and use of a compensation channel were adjusted from the default. No changes were made to resolution and slit width as the impact on this method was minimal. As seen in Table 6 and Figures 1 and 2, there was a 7x increase in the USP S/N ratio when the optimized detector settings were applied.

	Data rate (Hz)	Filter time constant	Resolution (nm)	Compensation	Slit Width (µm)	Noise (µAU)	USP S/N
Default	10	Normal	4	Off	50	70	7
Optimized	2	Slow	4	On (310–410 nm)	50	8	51

Table 6. Comparison of results using default and optimized settings.

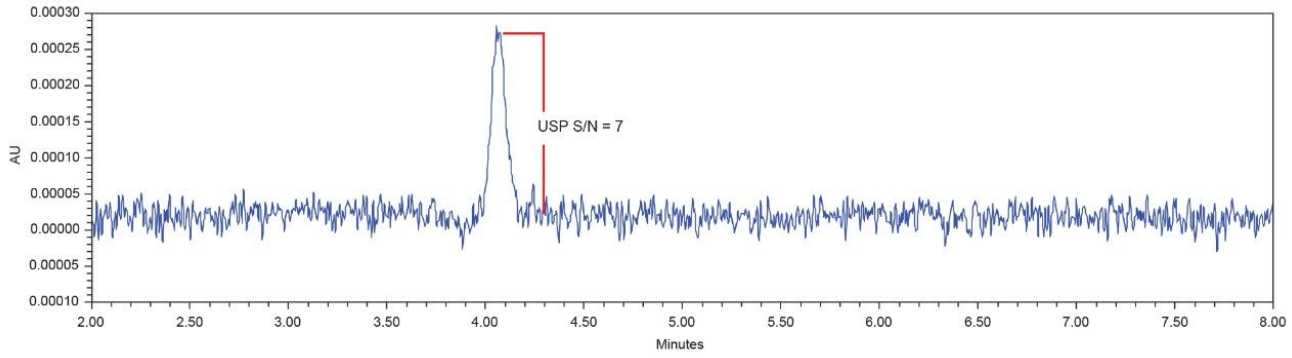

Figure 1. USP S/N with default detector settings.

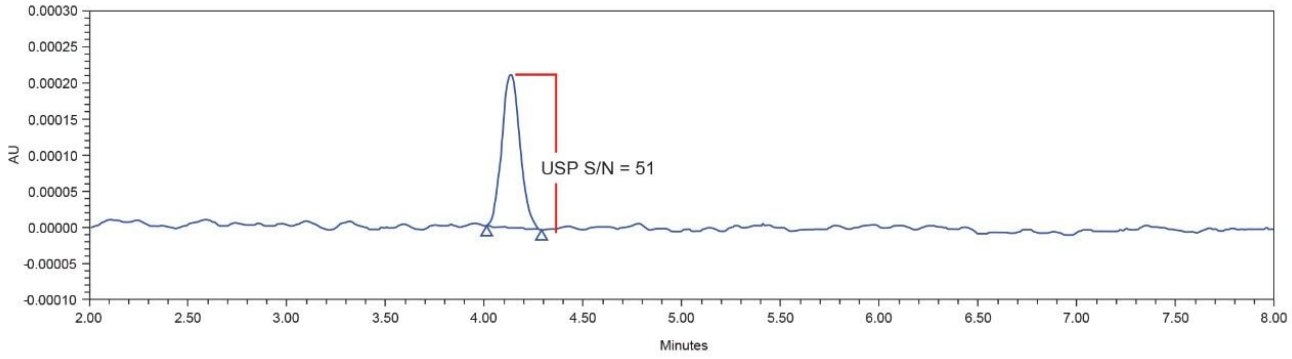

Figure 2. USP S/N with optimized detector settings.

Conclusion

While default PDA instrument method settings may be suitable for routine HPLC analysis, they may need to be adjusted to produce optimal separations. This study demonstrated how the Alliance iS HPLC System with PDA Detector allows the user to easily modify PDA settings to obtain optimal separations. Using the USP method for organic impurities in ibuprofen tablets, the data rate, filter time constant, slit width, resolution, and absorbance compensation settings were optimized. The optimized settings produced a 7-fold increase in sensitivity over that obtained using default values.

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720008901, June 2025