IMPACT OF DETECTOR SAMPLING RATE ON SIGNAL TO NOISE RATIO DURING METHOD MIGRATION

Waters™

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INTRODUCTION

While USP monographs provide important details of analytical methods to execute an assay, certain parameters such as detector sampling rate are generally not included. Such parameter can impact system performance and needs to be defined by the laboratory for each specific assay. This is especially important for sensitivity samples, for which signal to noise is becoming a key system suitability parameter to prevent impurities from going undetected and unreported.

For this work, the USP monograph for Organic Impurities in Acetaminophen tablets, a gradient LC method, was used to assess the impact of sampling rate on signal-to-noise (s/n) requirement for the sensitivity solution. In addition, a variety of HPLC systems, including the Alliance™ iS HPLC System, were also evaluated to assess the impact of detector characteristics when moving methods across systems.

System	Sampling Rate Options (Hz)
Waters System 1	1, 2, 5, 10, 20, 40, 80
Waters System 2	1, 2, 5, 10, 20, 40, 80
Alliance iS System	1, 2, 5, 10, 20, 40, 80, 160
Comparable System 1	1.25, 2.5, 5, 10, 20, 40 ,80, 120
Comparable System 2	1, 10, 50, 100
Comparable System 3	0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 125

Table 1. Typical sampling rate options

METHODS

Method Conditions

Column: Waters™ Atlantis™ T3 Column, 3 µm, 4.6 x 150 mm (P/N: 186003729)
Column Temp: 40 °C
Sample Temp: 10 °C
Injection Volume: 25 µL
Detection: 272 nm
Flow Rate: 0.9 mL/min
Run Time: 15 minutes
Buffer: 1.9 g/L Ammonium Formate in Water. Add 1 mL of Formic Acid to each 1 L of solution.
Diluent: Combine 50mL of Methanol and 950 mL of Buffer.
Mobile Phase A: 3.1 g/L Ammonium Acetate in Water. Add 1 mL of Trifluoroacetic Acid to each 1 L of solution.
Mobile Phase B: 3.1 g/L Ammonium Acetate in 10/75/15 of Acetonitrile/Methanol/Water. Add 1 mL of Trifluoroacetic Acid to each 1 L of solution.

Gradient Table

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
Initial	97.0	3.0
5	70.0	30.0
10	10.0	90.0
11	10.0	90.0
11.2	97.0	3.0
15	97.0	3.0

Standard:

Sensitivity Solution: 0.175 µg/mL of 4-Aminophenol in diluent.

Standard Solution: 0.175 µg/mL of 4-Aminophenol and 3.50 mg/mL of Acetaminophen in diluent.

RESULTS

Sensitivity solution was analyzed on three Waters systems with different detector sampling rates. Signal to noise ratio of 4-Aminophenol was calculated using Empower™ Software, shown in Table 2 and Figure 1.

System	1 Hz	2 Hz	5 Hz	10 Hz
Waters System 1	134.9	408.0	251.0	167.6
Waters System 2	77.3	217.3	133.9	101.6
Alliance iS System	113.3	92.1	73.2	58.6

Table 2. s/n data from Waters systems

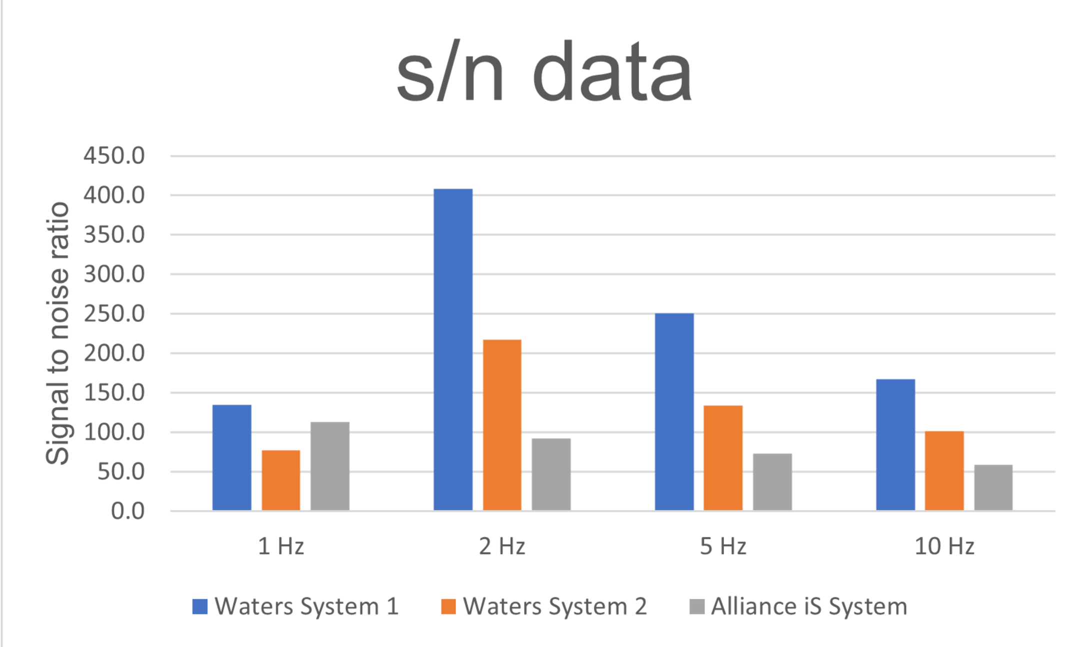

Figure 1. s/n data from three Waters systems

Standard solution was analyzed on three Waters systems with different detector sampling rates. System suitability was evaluated by calculating the RSD% of peak area of 4-Aminophenol and Acetaminophen, shown in Table 3.

Sampling Rate	1 Hz	2 Hz	5 Hz	10 Hz
Waters System 1				
4-Aminophenol	3.8%	0.10%	0.34%	0.35%
Acetaminophen	0.30%	0.35%	0.036%	0.32%
Waters System 2				
4-Aminophenol	2.0%	0.25%	0.22%	0.27%
Acetaminophen	0.075%	0.030%	0.090%	0.05%
Alliance iS System				
4-Aminophenol	0.11%	0.24%	0.26%	0.11%
Acetaminophen	0.14%	0.13%	0.034%	0.039%

Table 3. System suitability data (peak area RSD%) from Waters systems

For each of the data sets from the three Waters systems average points across peak values were pulled and summarized in Table 4.

Sampling Rate	1 Hz	2 Hz	5 Hz	10 Hz
Waters System 1	22	39	91	179
Waters System 2	24	39	82	164
Alliance iS System	21	41	100	220

Table 4. Points across peak data from Waters systems

Data file size was evaluated using results from Alliance iS HPLC system and summarized in Table 5.

Sampling Rate	1 Hz	2 Hz	5 Hz	10 Hz
Data File Size (KB)	24.3	31.3	52.4	87.5
Sampling Rate	20 Hz	40 Hz	80 Hz	160 Hz
Data File Size (KB)	157	298	579	1110

Table 5. Data file sizes from Alliance iS Sytem.

The sensitivity solution was also analyzed on three comparable systems using the same method with different detector sampling rate. Signal to noise ratio of 4-Aminophenol was calculated using Empower, and the results are shown in Table 6 and Figure 3.

Sampling Rate	1.25 Hz	2.5 Hz	5 Hz	10 Hz
Comparable System 1	31.2	76.5	67.5	56.6
Sampling Rate	1 Hz	10 Hz	50 Hz	100 Hz
Comparable System 2	35.0	168.4	68.2	45.9
Sampling Rate	1 Hz	2 Hz	5 Hz	10 Hz
Comparable System 3	198.8	186.7	108.4	99.5

Table 6. s/n data from three Comparable systems

RESULTS

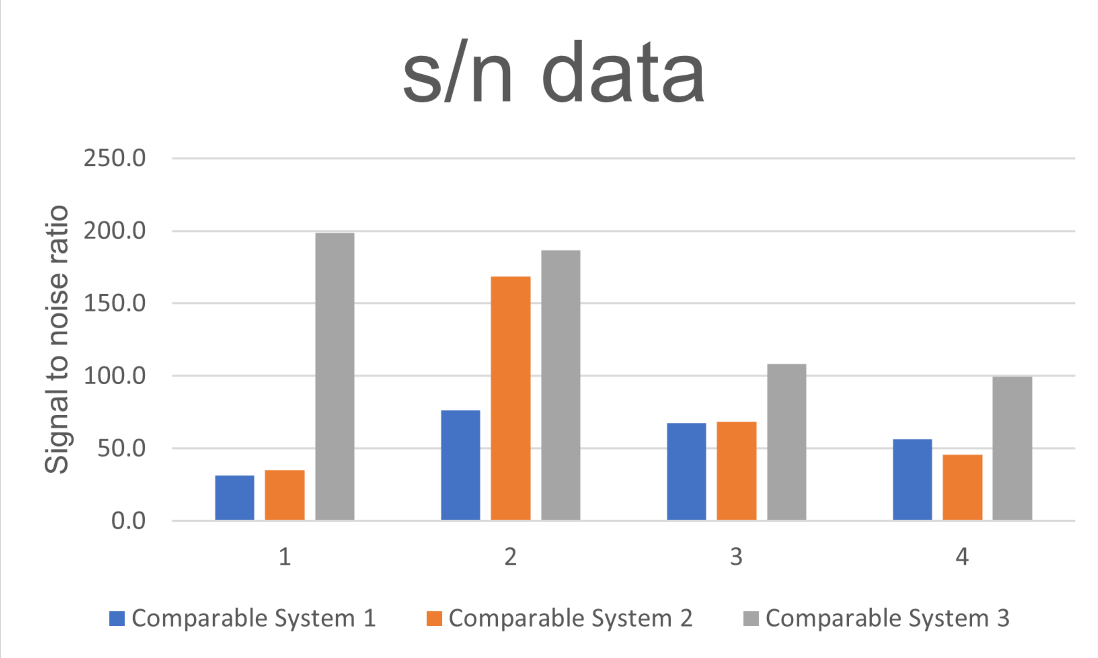

Figure 2. s/n data from Comparable Systems

DISCUSSION

While numerous instrument characteristics can impact method sensitivity and signal-to-noise ratio, detector sampling rate is a critical parameter. Higher detector sampling rate does not always provide higher signal-to-noise ratio as seen in Table 1 and Figure 1. For this method on Waters Systems with a 2489 detector, the optimum signal to noise ratio values were obtained using a sampling rate of 1 - 2 Hz. Increased sampling rate beyond 2 Hz increased detector noise which ultimately decreased the observed s/n.

To evaluate the impact of sampling data rate on system suitability of the method, the RSD% of the peak area was determined for the standard solution as required by the USP monograph. All RSD% values < 5% (Table 3), indicating system suitability was not impacted by sampling rates.

As a rule of thumb 25-50 points across a peak is required for reproducible quantitation. Analysis of points across peak data (Table 4) shows for this method, at 2 Hz, the points across the peak is within the 25-50 points range. However, if the detector sampling rate is lower than 1 Hz, not enough points across the peak would be achieved, which impacts quantitation.

Alternatively when detector sampling rate is set to be above the optimum value (≥ 5 Hz), greater noise is produced, resulting in decreased sensitivity. Higher detector sampling rate also generates larger data file size as shown in Table 5, which may impact data storage, backup and processing time.

Three comparable systems were evaluated using the same approach as the three Waters systems, producing comparable signal-to-noise results. It is worth noting that comparable system 2 demonstrated much lower signal-to-noise ratio at 1 Hz compared to 10 Hz. Reviewing the chromatographic results indicated peak distortion at the lower sampling rate as compared to chromatographic performance at higher sampling rates. Such peak distortion was not observed on the Alliance iS HPLC System.

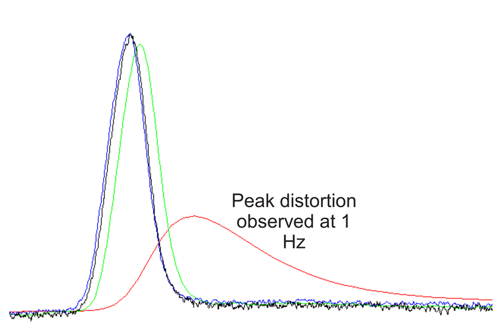

Figure 5. Overlay chromatogram from Comparable System 2.

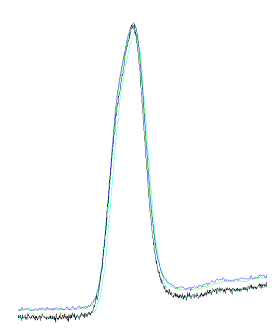

Figure 6. Overlay chromatogram from Alliance iS System.

CONCLUSION

- **Detector sampling rate significantly impacts s/n particularly for sensitivity samples.**
- **If no guidance is provided for regulated methods, the optimum detector sampling rate can be determined by evaluation of points across the peak and s/n.**
- **When moving a method with a sensitivity sample across different systems, detector sampling rate should be evaluated, particularly if no equivalent data rate is available.**

References
USP. Acetaminophen Tablets Impurities. DOI: https://doi.org/10.31003/USPNF_M200_05_01

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