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

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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 ratio 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 ratio requirement for the sensitivity solution. Alliance™ iS HPLC System and a comparable HPLC system were evaluated to assess the impact of detector characteristics.

System	Sampling Rate Options (Hz)
Alliance™ iS System	1, 2, 5, 10, 20, 40, 80, 160
Comparable System	1.25, 2.5, 5, 10, 20, 40 ,80, 120

Table 1. 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: 1.75 µg/mL of 4-Aminophenol and 3.50 µg/mL of Acetaminophen in diluent.

RESULTS

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

Sampling Rate	1 Hz	2 Hz	5 Hz	10 Hz	20 Hz	40 Hz	80 Hz	160 Hz
Signal to noise ratio	113.3	92.1	73.2	58.6	34.0	33.0	20.0	14.3

Table 2. s/n data from Alliance iS HPLC System

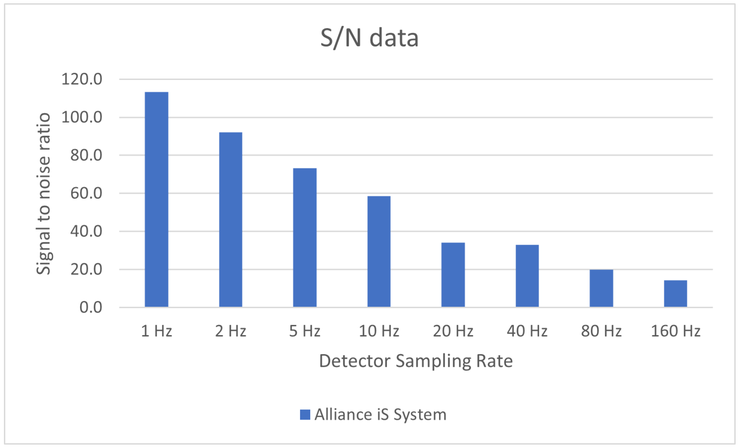

Figure 1. s/n data from Alliance iS System.

Standard solution was analyzed on Alliance iS HPLC System. 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
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 Alliance iS System.

Average points across peak values from Alliance iS HPLC system were pulled and summarized in Table 4.

Sampling Rate	1 Hz	2 Hz	5 Hz	10 Hz
Points across peak	21	41	100	220
Sampling Rate	**20 Hz**	**40 Hz**	**80 Hz**	**160 Hz**
Points across peak	395	792	1548	2816

Table 4. Points across peak data from Alliance iS System.

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 System.

The sensitivity solution was also analyzed on a comparable HPLC system at sampling rate of 10 Hz and lower. Signal to noise ratio of 4-Aminophenol was calculated using Empower, and the results are shown in Table 6. Data comparison of Alliance iS system and the comparable system are shown in Figure 3.

Sampling Rate	1 Hz	2 Hz	5 Hz	10 Hz
Signal to noise ratio	31.2	76.5	67.5	56.6

Table 6. s/n data from a comparable system.

RESULTS

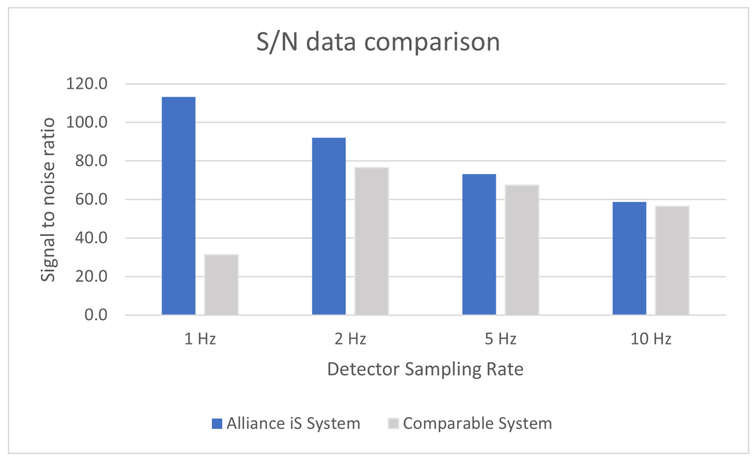

Figure 2. s/n data comparison of Alliance iS HPLC System and a comparable system.

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 2 and Figure 1. For this method on the Alliance iS HPLC System, 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 thumbs, 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 may impact 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.

A comparable system was evaluated using the same approach as the Alliance iS HPLC system. Similar trend was observed. Lower sampling rates produced higher signal to noise results. When compared to the comparable system at the optimum sampling rate range Alliance iS HPLC system produced better signal to noise results (Figure 2).

CONCLUSION

- **Detector sampling rate significantly impacts signal to noise ratio, 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 signal to noise ratio.**
- **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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