

Application Note

High Throughput Analysis for Adenosine Injection USP

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Abstract

This application note summarizes the use of the Alliance HPLC System and *IS* Columns for a rapid and effective assay of adenosine injection USP.

Introduction

Adenosine is an endogenous chemical involved in a wide range of metabolic processes, such as energy metabolism and inflammation.

At pharmaceutical doses, injected adenosine can effectively treat certain types of cardiac arrhythmias. With a half-life of less than 10 seconds in the blood, this is a fast-acting and fast-clearing drug.

Fast chromatography is a High Performance Liquid Chromatography (HPLC) technique using short columns packed with small particles to deliver rapid separation of simple mixtures that is desired. The Waters Alliance HPLC System with Waters Intelligent Speed (*IS*) Columns delivers excellent performance for fast chromatography. The Alliance HPLC System can perform an assay at higher flow rates and lower backpressures, increasing analytical throughput while meeting or exceeding assay acceptance criteria.

This application note summarizes the use of the Alliance HPLC System and *IS* Columns for a rapid and effective assay of adenosine injection USP.

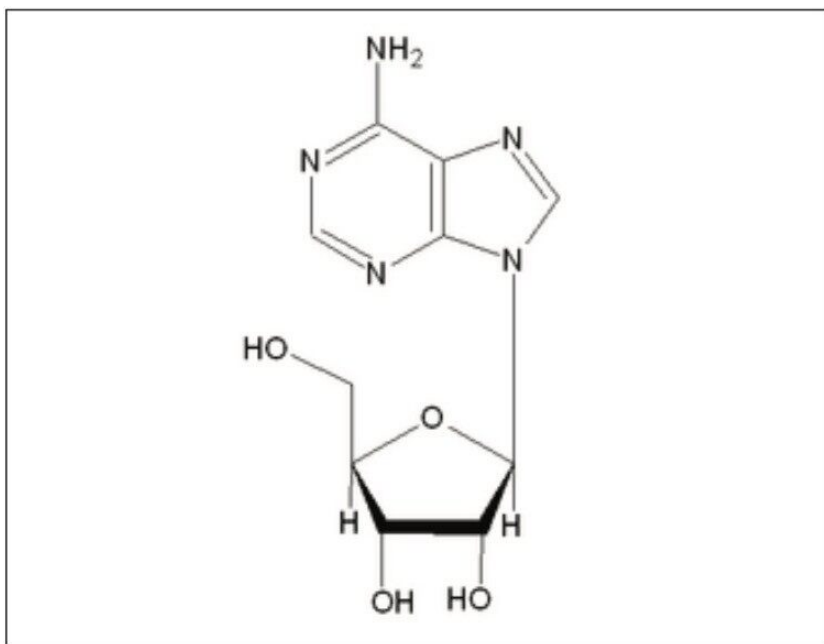


Figure 1. Structure of adenosine.

Experimental

LC Conditions

LC system:	Alliance e2695 Separation Module
Column:	Atlantis IS 4.6 x 20 mm dC ₁₈ , 3μm
Column temperature:	40 °C
Sample temperature:	15 °C
Injection volume:	2 μL
Mobile phase:	10 mM sodium phosphate, 5 mM tetrabutyl ammonium phosphate, 2% acetonitrile in water

	(isocratic)
Flow rate:	3.0 mL/min
Run time:	2.5 min
Detection:	Waters 2998 Photodiode Array (PDA) Detector
PDA wavelength:	254 nm at 1.2 nm bandwidth
Date rate:	10 Hz, filter time constant: "fast" (0.1 s)

The Alliance HPLC System was plumbed with 0.005" PEEK tubing pre-column and post-column to the 2998 PDA Detector to minimize band broadening. The sensitivity, suitability, adenosine standard, and sample test solutions were made according to the monograph for Adenosine Injection USP.¹ The run time of the analysis was based upon two and a half times the retention time of the adenosine peak from the analysis of the assay sensitivity solution, as per the USP monograph.

Results and Discussion

The instrumental parameters for this Alliance HPLC System/*IS* Column assay developed from the original HPLC method. The original USP method called for a 3.9 mm x 30 cm, 10 µm L1 HPLC column, a flow rate of 2.5 mL/min, and an injection volume of 10 to 20 µL. Limited information concerning the original assay indicated a relatively short runtime, 6 minutes, with low retention and marginal resolution. A modern upgrade of the method was sought.

The short run-time and higher flow rate made this analysis conducive to an *IS* Column-based method. To that end, an Atlantis T3 *IS* 4.6 x 20 mm Column replaced the 3.9 x 300 mm HPLC Column, and the flow rate was increased to 3.0 mL/min, the classic *IS* Column flow rate. The 2 µL injection volume was determined by experiment to give the best sensitivity without causing column overload. These three simple changes delivered an assay that far exceeded the USP acceptance criteria (Table 1).

USP acceptance criterion	Adenosine using IS Columns
Resolution ≥ 6.0	8.0
USP Tailing ≤ 2.0	1.0
% RSD Adenosine Area $\leq 1.5\%$	0.2%

Table 1. Comparison of the mean adenosine IS Column assay results to USP acceptance criteria.

In overlay, the peaks of the assay suitability solution were well resolved and quite symmetrical (Figure 2). The nucleoside inositol was used here as a resolution standard. The mean USP resolution of the inositol and adenosine peaks was 8.0, much greater than the 6.0 required by the USP. The mean tailing factor of the adenosine and inositol was 1.0 and 1.1, respectively. Again, this was far superior to the acceptance criterion of 2.0. With a % RSD for area precision of 0.2%, this assay is highly repeatable. The retention time precision of 0.2% RSD was not reported in the table.

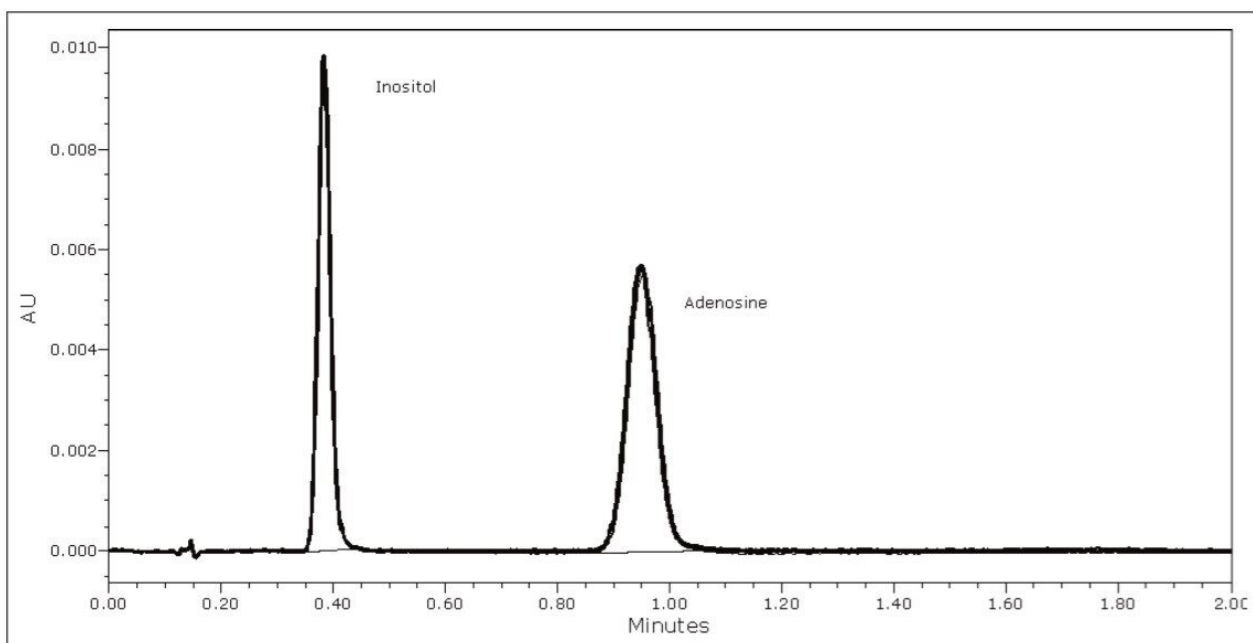


Figure 2. Overlay chromatogram of six injections of the suitability solution of adenosine injection USP using IS Columns.

The overlay of six chromatograms of an assay test solution (Figure 3) also shows the excellent retention time and area repeatability of this analysis.

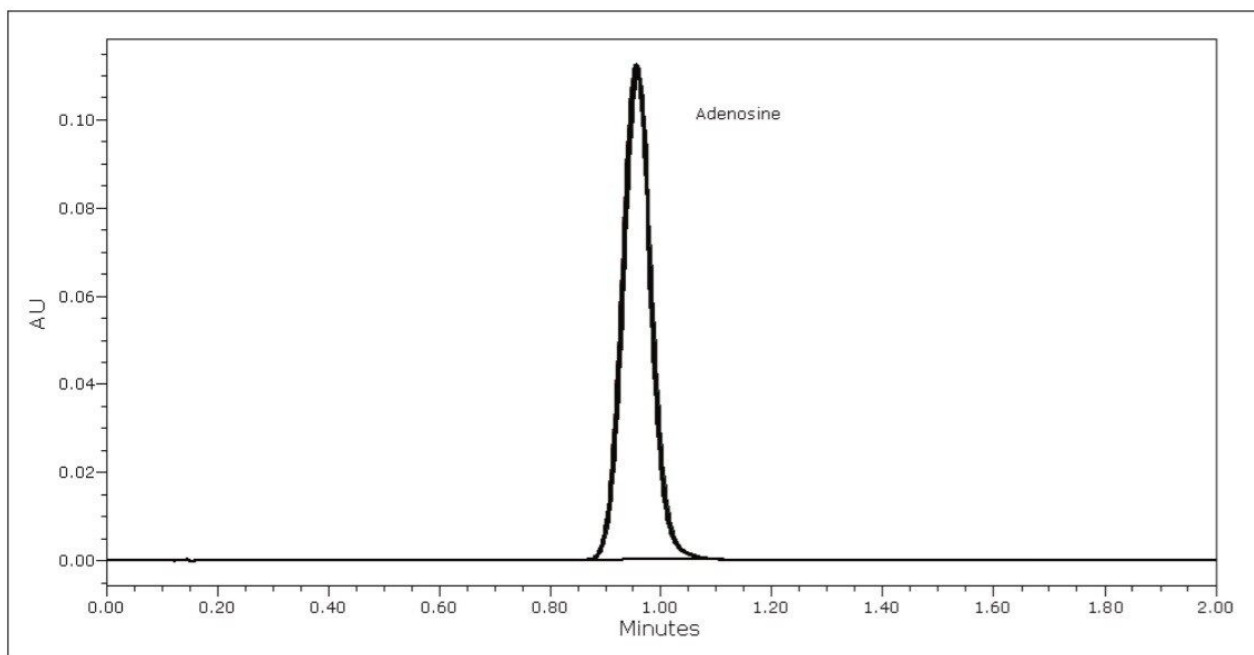


Figure 3. Overlay chromatogram of six injections of an adenosine test solution.

Conclusion

- This Alliance HPLC System and Intelligent Speed Column method was smoothly developed by simple changes that preserved the mobile phase and solution preparations of the USP monograph.
- The excellent injection repeatability and pump performance of the Alliance HPLC System yielded superior results that easily met the precision acceptance criteria for this assay.
- Coupling the Alliance HPLC System to the Atlantis T3 /S Column delivered high throughput for the analysis of adenosine and increased the chromatographic quality.
- This high-quality, high-throughput assay will make any subsequent method verification a rapid and successful process.

References

1. The United States Pharmacopeia USP 31, The National Formulary NF 27 United States Pharmacopeial Convention, Inc. 2008, pg 1433.

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Alliance HPLC System <<https://www.waters.com/534293>>

2998 Photodiode Array (PDA) Detector <<https://www.waters.com/1001362>>

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