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Development and Validation of Adenosine by RP-HPLC Method in Bulk drug and Pharmaceutical dosage forms

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ABSTRACT

A simple, economic, selective, precise and accurate High-Performance liquid Chromatographic method used for the estimation of Adenosine in bulk drug. The mobile phase used was of Mixture of Acetonitrile and water in the proportion 5:95 respectively. This Mobile phase was allowed to flow at rate of 0.8ml/min. And this was found to give a sharp peak of Adenosine at a retention time of 3.78 min. Analysis of HPLC for Adenosine was carried out at a wavelength of 256 nm. Linear regression analysis data for the Calibration curve showed a good linear relationship, in concentration range of 50-100ppm and regression coefficient 0.991. The linear regression equation was Y=71258× the developed method was employed with a high degree of precision and accuracy for the analysis of adenosine. The inter and intraday variation was less than 2%. The mean recovery of the drug was 99.39%. The proposed method is simple, fast, accurate, and reproducible hence, it can be applied for routine quality control analysis of Adenosine.

Keywords: RP-HPLC, Adenosine, precision, accuracy.

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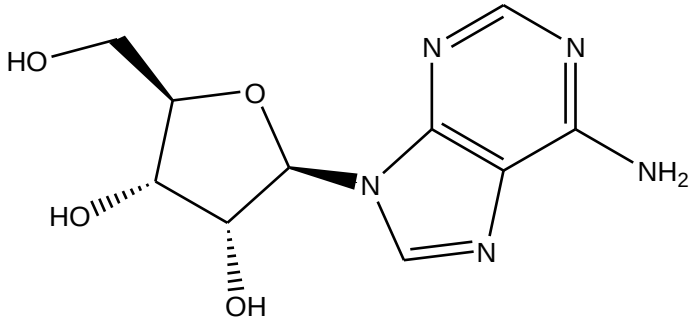
INTRODUCTION

Adenosine is a short acting molecule that is a purine nucleoside having half-life is 6 seconds. Adenosine receptor activation results in multiple different actions depending on the location. Adenosine plays a major role in energy transfer (adenosine triphosphate or ATP) and in cellular signalling (cyclic AMP).The uses of adenosine relate to its ability to block the AV Node. Giving a 6 mg IV bolus followed by a saline flush can be helpful during a narrow complex tachycardia. This can terminate atrial tachycardia as well. Adenosine will not terminate atrial fibrillation and atrial flutter allowing an accurate diagnosis to be made .



(2R,3R,4S,5R)-2-(6-aminopurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol

Analysis of adenosine has mainly been accomplished by different methods such as infrared spectroscopy, GC, HPLC methods were more frequently employed for the analysis in various environmental samples. However no reported RPHPLC method for the analysis of Adenosine in its technical grade with the specified mobile phase is mentioned. This chapter describes a validated RP-HPLC method for the quantitative determination of adenosine. The author has developed RP-HPLC method based on the use of C18 column, without use of any internal standard. An attempt has been made to develop and validate all methods to ensure their accuracy, precision, and other analytical method validation parameters as mentioned in the various guidelines.

MATERIALS AND METHOD:

An isocratic HPLC (shimadzu HPLC) with one LC-10 AT VP pumps, with UV/VIS detector, and a hypersil C-18 Column 250 mm x 4.6 mm i.d. particle size 5 µm was used.

Reagents and Chemicals

All the chemicals used were of HPLC grade and A.R. grade. Double Distilled water was used for making the solutions. The commercially available Adenosine tablets were procured from the local market.

Chromatographic Conditions

The content of the mobile phase was Acetonitrile and water in the ratio (5:95v/v). The mobile phase was filtered through 0.45 µm membrane filter and sonicated for 10 min. The flow rate of the mobile phase was maintained at 0.8 ml/min. The column temperature was set at 40°C and the detection was carried out by UV-detector wavelength at 256 nm. The run time was set at 5 min and the volume of the injection loop was 20 µL. Prior to injection of the drug solution, the column was equilibrated for 40 min with the mobile phase flowing through the system.

Procedure

Stock solution of Adenosine was prepared by dissolving 100 mg of Adenosine in 100 ml standard volumetric flask which had approximately 50 ml of mobile phase and the solution was sonicated for 20 min and then the volume was made upto the mark with MP to obtain a concentration of 1000 µg/ml. Subsequent dilutions of this solution were made with mobile phase to obtain the concentration range of 50-100 µg/ml. The standard solutions prepared as above were injected into the 20 µL loop and the chromatogram was recorded and shown in Figure 2

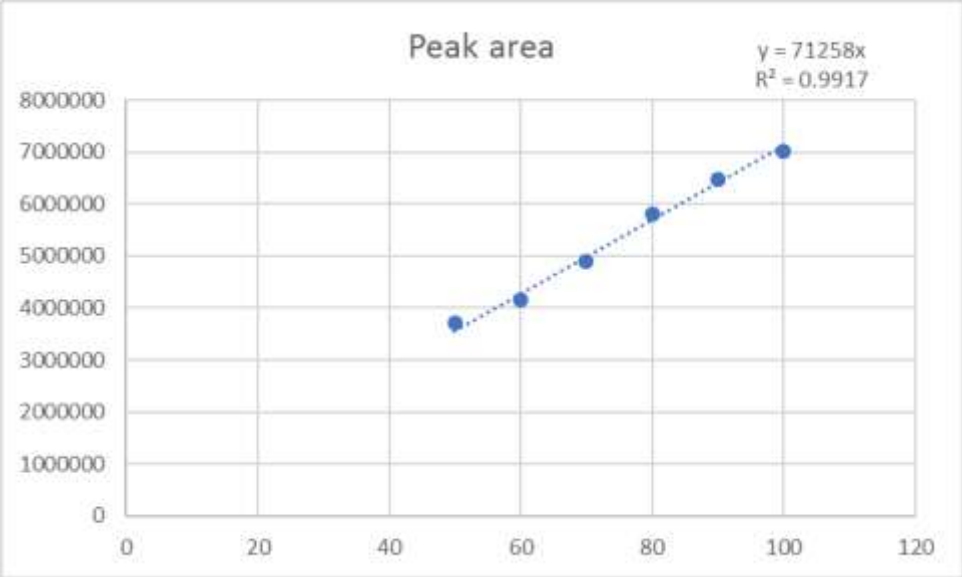


Figure 2: Calibration curve of adenosine

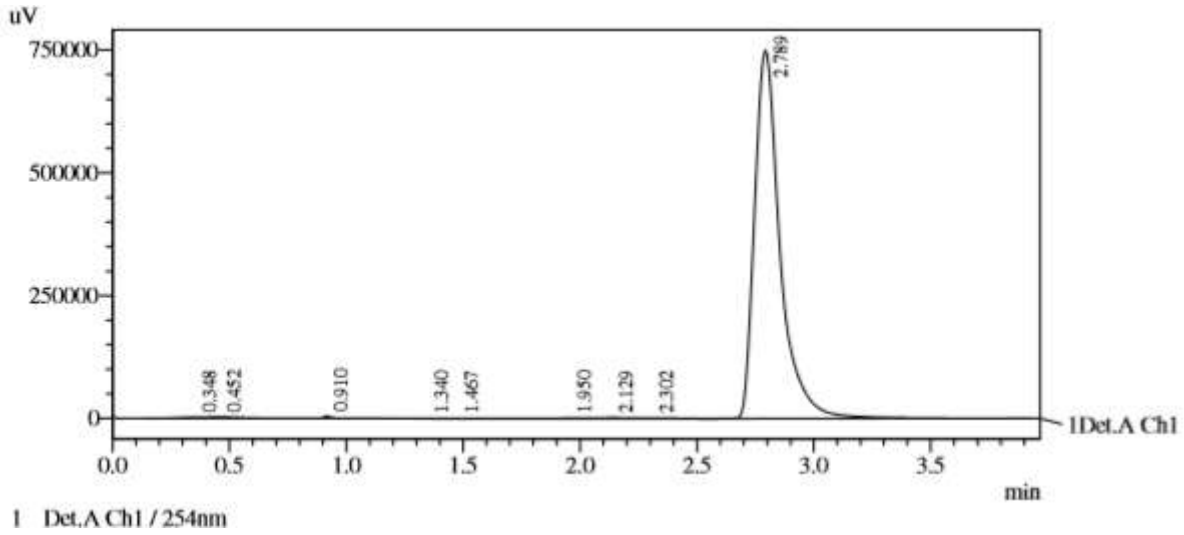


Figure 3: Chromatogram of Adenosine

The retention time of Adenosine was found to be 2.78 min. The calibration curve was obtained by plotting concentration against peak area ratio. The calibration curve was found to be linear and shown in Figure 3. The amount of adenosine present in sample was calculated through the standard calibration curve. The linearity experiment was carried out in triplicate to ensure accuracy and precision of the method.

Table 1: Assay of adenosine

Drug	Mean	Std dev.	Coefficient of variation
Adenosine	99.54	0.150041	0.1247

Table 2: Precision of proposed HPLC method

Concentration ug/ml	Mean		Std Dev.		Coefficient of variation		Std error	
	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day
60	37.76	37.66	0.142	0.134	0.3451	0.2151	0.0704	0.0341
70	58.98	58.91	0.153	0.198	0.1995	0.0814	0.0786	0.0356
80	79.42	79.31	0.083	0.043	0.0565	0.0553	0.0312	0.0765

Assay

25 tablets each containing 250 mg of adenosine API was weighed accurately and powdered. A quantity equivalent to 100 mg of API was weighed and transferred to 100 ml volumetric flask containing 50 ml of mobile phase. The contents were sonicated for 15 min and volume was made upto the mark with the mobile phase. The solution was filtered through a membrane filter. The solution obtained was then allowed to dilute with the mobile phase so as to acquire a concentration of 1000 µg/ml. Sample solution was also injected under the same conditions and the chromatogram was recorded in triplicate. The amount of Adenosine present in tablet formulation

was determined by comparing the peak area from the standard. The results are furnished in Table 2.

Linearity

The standard curve was obtained in the concentration range of 20-100 µg/mL. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits. Acceptance criteria: Correlation coefficient should be greater than or equal to 0.999.

Precision

The precision was assessed in terms of intra-day, inter-day variation. The variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.). The results are in Table 2.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

The LOD and LOQ were predicted based on the parameters of standard error of estimate and slope, calculated from linearity of the response data of adenosine.

Robustness

The robustness was tested by changing the flow rate to 0.8 and 1.2ml/min.

Accuracy

The accuracy of the HPLC method was checked by adding known amount of standard drug solution to a pre-analysed formulation. The recovery studies were carried out in triplicate. The accuracy was in terms of recovery at three levels 80%, 100% and 120%. The results are furnished in Table 3.

Table 3: Recovery studies of HPLC

Sr no.	List of % recovery	Mean	Std. Dev.	Co-efficient of variation
1	80	99.6632	1.0653	1.0542
2	100	99.4322	0.1342	0.1369
3	120	99.5893	0.3532	0.3953

Table 4: System suitability parameters

Parameters	RP-HPLC method
Linearity range (µg/ml)	50-100ug/ml
Regression coefficient (r^2)	0.9917
Limit of Detection (µg/ml)	0.3976
Limit of Quantification (µg/ml)	1.3215
Retention time (min)	2.789
Tailing factor	1.616
Theoretical plate	4990

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions were carried out with various combinations of buffer a methanol and by observing the peak parameters, the run time of the method was set at 5 min, Adenosine appeared on the typical chromatogram at 2.789min, which indicates a good base line. When the drug solution was injected 3 times, the retention time of the drug was same. Linearity range was in the concentration range of 50-100 µg/ml. The regression equation of Adenosine concentration over its peak area ratio was found to be Y = 71258X + 1751 (r=0.9917) where Y is the peak area ratio and X is the concentration of Adenosine (Fig. 3). The proposed HPLC method was validated for precision (intra-day and inter-day) variation. The coefficient of variation in the peak area of the drug for 3 replicate injections was found to be less than 2%. The tailing factor was found to be 1.6, which indicates good shape of peak. The number of theoretical plates was found to be 4990, which shows efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.3976 µg/ml and 1.3215µg/ml which indicates the sensitivity of the method. The use of acetonitrile and water in the ratio of 05:95 v/v resulted in peak with good shape and resolution. The high percentage of recovery of Adenosine ranging from 99.43-99.66 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION

The given HPLC method was found to be simple, sensitive, precise and accurate for the estimation of Adenosine in pharmaceutical formulations. Hence, this method can conveniently be adopted for routine quality control analysis of Adenosine in bulk dosage form.

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