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Area under Curve and First Order Derivative Spectrophotometric Method and Development and Validation of Adenosine in Bulk drug

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Abstract : The main aim of the study was to develop and validate simple, sensitive, precise and cost-effective method for the estimation of Adenosine in bulk and pharmaceutical dosage form as per ICH guidelines. Two spectrophotometric methods have been developed for determination of Adenosine from tablet dosage form. First method was area under curve method in which the range of 251.28–260.88 nm was selected. Second method was first order derivative spectrophotometric method which had absorbance measured at λ_{min}= 243.99 nm, λ_{max} = 268.62nm and Zero cross = 256.30 nm. The calibration curves were plotted for the method by using instrumental response at selected wavelengths and concentrations of analyte in the solution. Linearity for detector response was observed in the concentration range of 10-18µg/ml at the λ_{max}= 256.89 nm. The method was validated by the International Conference on Harmonization using parameters of Accuracy, Precision, LOD and LOQ. Good reproducibility and recovery was observed in both the above methods with % RSD less than 2. The proposed methods were found to be rapid, specific, economical and accurate and can be successfully applied for the routine analysis of Adenosine in bulk and pharmaceutical dosage forms.
Keywords : Accuracy, Linearity, Precision, Adenosine, First order derivative spectroscopy, Area under curve method.

Introduction:

Adenosine is (2R,3R,4S,5R)-2-(6-aminopurin-9-yl)-5-(hydroxymethyl) oxolane-3,4-diol. Molecular formula is $C_{10}H_{13}N_5O_4$ and its molecular weight is 267.245 g/mol. Adenosine belongs to the class of antiarrhythmic drugs.It is used for the rapid treatment of supraventricular tachycardias[2,4]. It supresses the atrioventricular conduction making it very useful in treating paroxysmal supraventricular tachycardia**.**The administration of adenosine also reduces blood flow to coronary arteries past the occlusion[3,4,5]. Due of the effects of adenosine on AV node-dependent SVTs, adenosine is considered a class V antiarrhythmic agent.

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A detailed survey of literature revealed the estimation of Adenosine by RP-HPLC and spectrophotometer[1,2]. Several techniques have been reported for assay by quantitative determination of Adenosine in biological fluids which high performance liquid chromatography (HPLC), LC-MS in dosage forms[5,6]. This study established new, precise and reproducible spectrophotometric methods for quantification of adenosine from bulk and tablet dosage form.

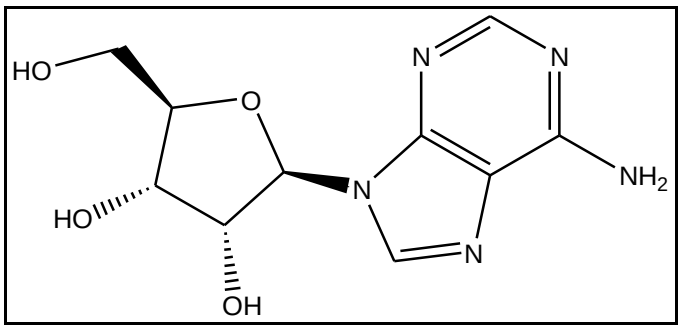

Figure 1: Structure of Adenosine

Materials and Methods:

A double-beam UV-Visible spectrophotometer, model UV1800 (Shimadzu, Japan) having twomatched cells with 1cm light path.A Citizen electronic balance (Sartorius) was used for weighing the samples.Calibrated volumetric glass wares (Borosil) were used in this study.Sonication of the solutions was carried out using an Ultrasonication. Adenosine pure drug was obtained fromMylan Laboratories Pvt. Ltd. Hyderabad (India). Commercially available tablets were procured from local pharmacy. Distilled water was used as solvent.

Method Development:

Preparation of Standard Solution:

Accurately weighed 10mg quantity of Adenosine was transferred into10 ml volumetric flask, to this 7ml of distilled water was added and sonicated until all drug gets dissolved. The concentration of 1000 µg/ml was obtained by adjusting the volume up to 10ml solution. Fromresulting solution 1ml solutionwas pipettedout into 100 ml volumetric flask and volume adjusted with distilled water to obtain10 µg/ml standard stock solution. This solution was further diluted with distilled water to obtain desired concentrations of working standard solutionsin the range of 10 – 18µg/ml[1].

Determination of Absorption Maxima

Accurately weighed Adenosine was dissolved in distilled water to obtain final solution of 10µg/ml. This working standard stock solution of Adenosine was scanned in the range of 200 to 400 nm against distilled water as a blank[1,6]. The maximum absorbance of solution was measured at the wavelength 256.89 nm (Figure 2).

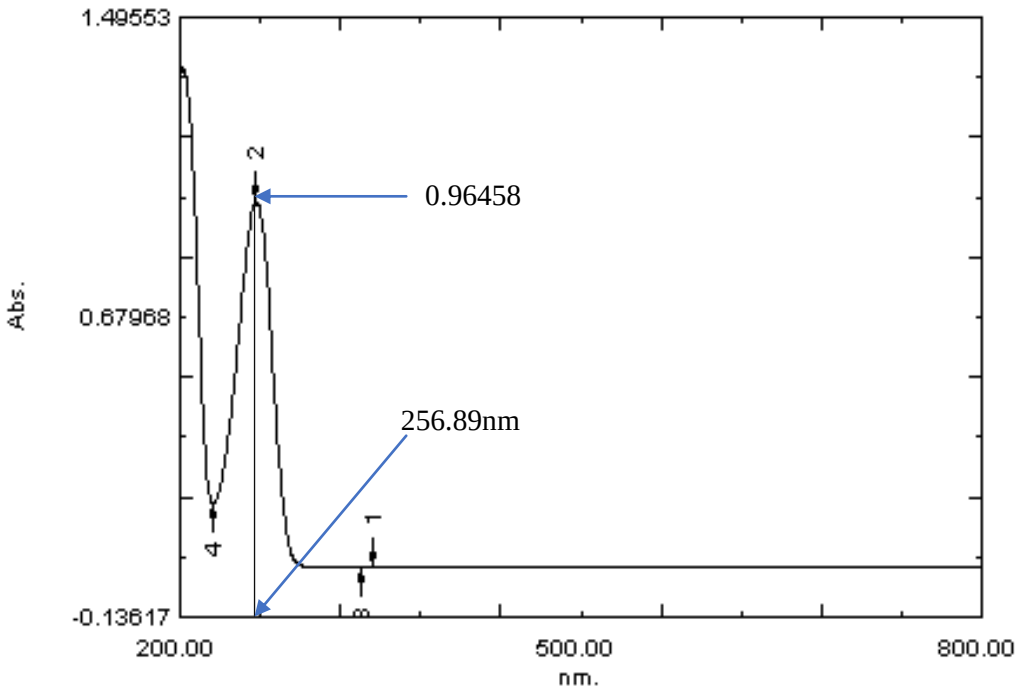

Figure 2: Determination of λ_{max} of Adenosine

From the standard stock solution fresh aliquots were pipette out and suitably diluted with distilled water to get final concentration which would be in the range of 10-18 (µg/ml). The solutions were scanned under 200-400 nm wavelength range and a sharp peak was observed at 256.89nm (figure 2). Calibration curve was plotted which had absorbance on y-axis and concentration of solution on x-axis (figure 5). The drug showed linearity in the concentration range 10-18µg/mL with a correlation coefficient value of 0.9982.

Area under curve method (Method A):

From the spectra of drug (Fig. 3), area under the curve in the range of 251.28-260.88 nm was selected for the analysis. The calibration curves for Adenosinewas prepared in the concentration range of 10-18µg/mL at their respective AUC range.Area calculation calculatestheareaboundedbythecurveandhorizontal axis. α is area of portion bounded by curve datai.e. a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1andλ2 are wave length are presenting start and end point of curve region.

Area calculation $(\alpha+\beta)=\lambda_1/\lambda_2 Ad\lambda$

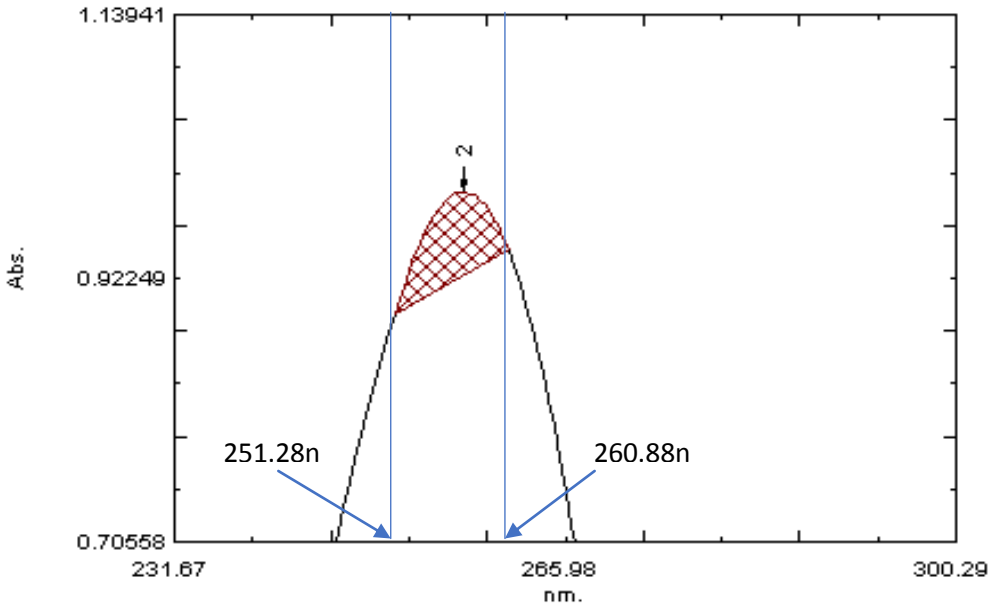

Figure3: Area under curve graph of Adenosine

Working standard solution of the drug was scanned within 400-200 nm after baseline correction and the spectral data was then processed to obtain first order derivative spectrum[1,2]. It was observed that Adenosine shows zero crossing at 256.30nm, Adenosine showed absorbance maxima at 268.62 nm and minima at 243.99nm. Hence 257.00 nm were selected as analytical wavelengths in this method for determination of Adenosine[8,9].

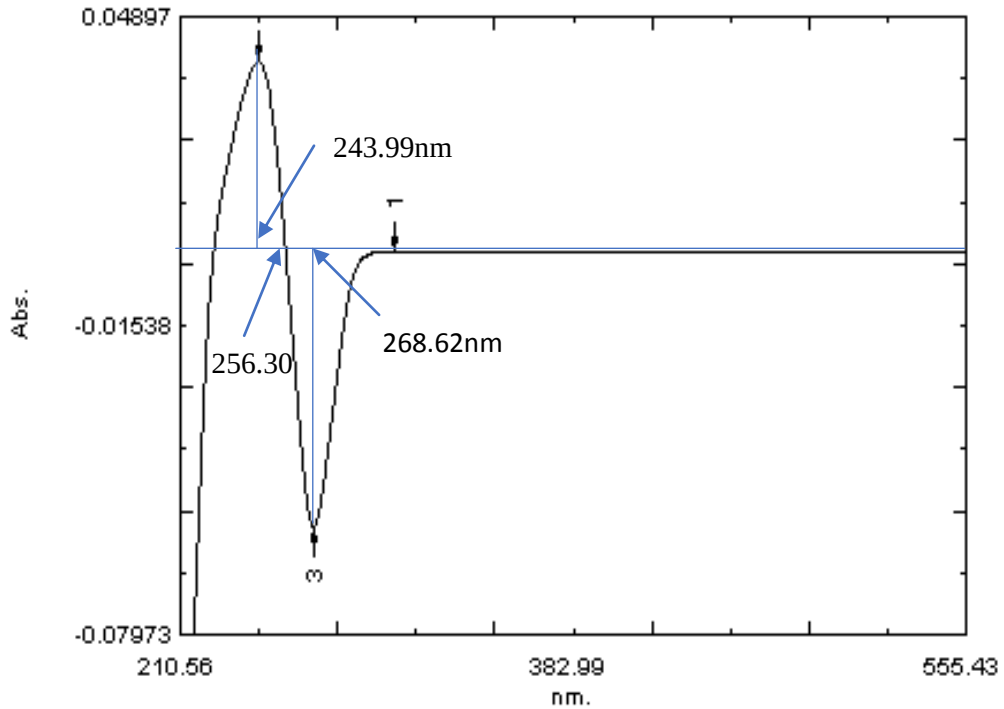

Figure 4: First derivative spectra of Adenosine

Validation of the developed method:

The aim of validation of analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline[7,11].

Linearity:

Fresh aliquots were prepared from the stock solution (1000µg/ml) in different concentrations. The samples were scanned in UV–visible spectrophotometer against reagent blank[7,8]. It was found that the selected drug shows linearity between the 10-18µg/ml (Table 1)

Table 1: Linearity results of Adenosine in Distilled water

Concentration (µg/ml)	Absorbance (nm) by Method A	Absorbance (nm) by Method B
10	0.60635	0.0061
12	0.73846	0.01355
14	0.8434	0.02447
16	0.96458	0.03155
18	1.10202	0.04255

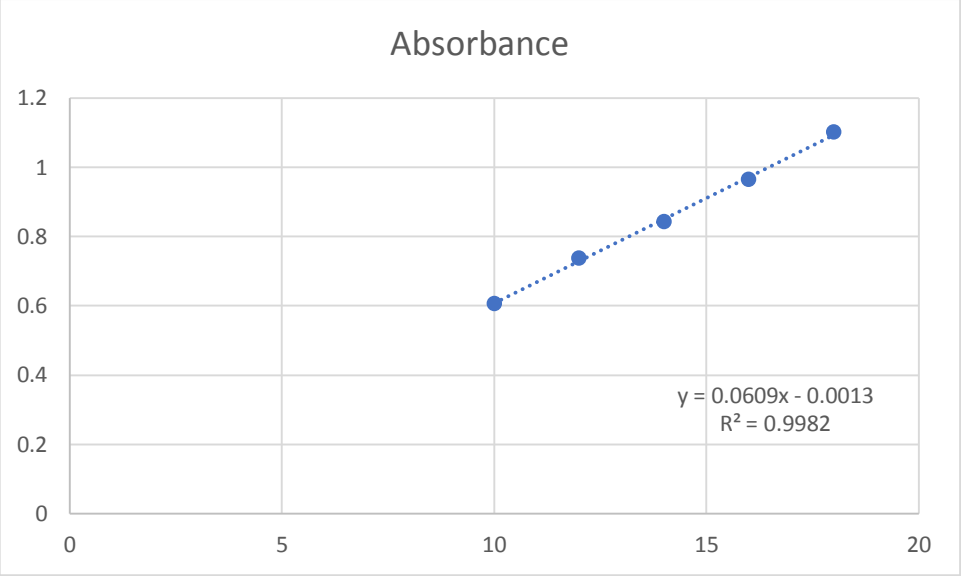

Figure 5: Calibration curve of Adenosine by AUC method

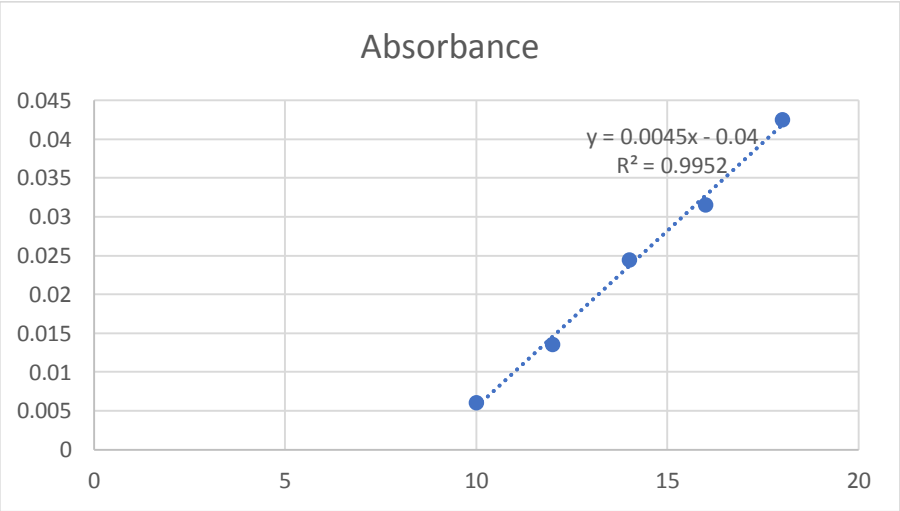

Figure 6: Calibration Curve of Adenosine by First order Derivative Method

Precision:

Repeatability:

Repeatability was checked by injecting (n=6) standard solutions of Adenosine (16µg/mL). Area under curve of each of these solutions was measured in the range of 251.28- 260.88 nm and absorbance at λ_{max}= 268.62nm, λ_{min}= 243.99nm and Zero cross = 256.30nm were measured for method-II[12,13,14]. Percentage relative standard deviation (%RSD) was calculated (Table 2).

Intermediate Precision (Reproducibility):

The intra-day and inter-day precision of the proposed method was determined by analysing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of Adenosine (12, 14 and16µg/ml). Relative standard deviation (%RSD) was recorded[12,13,14]. The results were tabulated in (Table 2)

Table 2: Results of Precision

Precision	**Method A AUC (%RSD)**	**Method B First order derivative(%RSD)**
Repeatability	0.6845	0.6122
Intraday	0.7648	0.6994
Interday	0.8987	0.8174

Accuracy (Recovery studies):

The accuracy for the analytical procedure was determined at 80%, 100% and120% levels of standard solution Absorbance was measured at 256.89 nm andresults were expressed in terms of % recoveries[9,10,11]. Standard deviation and % RSD were calculated. (Table 3)

Table No.3: Results for Accuracy of Adenosine

Test sample (µg/ml)	**Accuracy Level (%)**	**Mean % Recovery for Method A**	**%RSD for Method A**	**Mean % Recovery For Method B**	**%RSD for Method B**
	80	99.80	0.845	98.45	0.955
16 µg/ml	100	99.40	0.758	98.62	0.988
	120	98.54	0.699	99.41	0.757

Limit of Detection (LOD)

It is the minimum quantity of an analytethat can be detected and not necessarily determined, in a quantitative way. The formula used is; LOD=3.3 × S.D/Slope Where; S. D=Standard Deviation[8,9] (Table 4).

Limit of Quantization (LOQ)

It is the lowest concentration of an analyte in a sample that may be determined with accuracy and precision with acceptable value. It was calculated by the following formula; LOQ=10 × S.D/Slope[8,9] (Table 4).

Table 4: Results of LOD and LOQ

Method	Method A AUC	Method B First order derivative
LOD	0.4259	1.2554
LOQ	1.3455	3.2154

Result and discussion

The study was conducted to develop a simple and specific AUC and First Order Derivative spectrophotometric method for the determination of Adenosine in tablet dosage form. The generated regression equations were

Method A-$\int_{251.28}^{260.88} Ad\lambda = 0.0609x - 0.0013$ $R^2 = 0.9982$

Method B-$\frac{dA}{d\lambda}$ = 0.0045x - 0.04 R^2 = 0.9952

Where,$\int_{251.28}^{260.88} Ad\lambda$is area under curve between 251.28 to 260.88 nm, $\frac{dA}{d\lambda}$is amplitude difference, x is concentration and R^2 is correlation coefficient. The R^2 value as 0.9982 and 0.9952 indicate that developed methods were linear. The above methodswere found to be precise as % R.S.D valuesfor intraday and interday precision were satisfactory. The drug at each of the 80 %, 100 %and 120 % levels showed good recoveries (98.45 % to 99.80 %). Hence, it can be said that thesemethods were accurate. The LOD and LOQ were calculated as 0.4259 µg/ml and 1.3455µg/ml for Method-I and 1.2554µg/ml and 3.2154µg/ml for Method-II respectively. The methods can be used for the routineestimation of the Adenosine in bulk and tablet dosage forms. The validation parameters are summarized in Table 5.

Table No. 5: Optical Parameters/ Summary of Adenosine

Parameter	Results for Method A	Results for Method B
Range	251.28-260.88nm	243.99-268.62
Linearity range	10-12 µg/ml	10-12 µg/ml
Standard regression equation	y = 0.0609x - 0.0013	y = 0.0045x - 0.04
Correlation coefficient	R^2=0.9982	R^2=0.9952
Repeatability	0.6845	0.6122
Intraday	0.7648	0.6994
Interday	0.8987	0.8174
Accuracy (Mean % Recovery)	99.24%	98.82%
LOD	0.4259	1.2554
LOQ	1.3455	3.2154

Conclusion

The two spectrophotometric methods were developed. The proposed method was simple, accurate, rapid and precise for the estimation of Adenosine in bulk and pharmaceutical dosage forms.Hence, it can be effectively applied for the routineestimation of Adenosine in bulk and marketed formulation.

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