METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ADEFOVIR DIPIVOXIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS AND ITS STRESS DEGRADATION STUDIES USING UV-VIS SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Objective: To develop simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of adefovir, an anti-HIV drug, in bulk and pharmaceutical dosage form.

Method: The solvent used was methanol and the λmax or the absorption maxima of the drug was found to be 260 nm. Adefovir dipivoxil was subjected to stress degradation under different conditions recommended by ICH. The samples so generated were used for degradation studies using the developed method.

Results: A linear response was observed in the range of 5- 40μg/ml with a regression coefficient of 0.999. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. The stress degradation studies showed that Adefovir undergoes degradation in acidic and alkaline conditions whereas it is relatively stable when exposed to dry heat, oxidation and photolytic conditions.

Conclusion: This method can be used for the determination of Adefovir in quality control of formulation without interference of the excipients.

Keywords: Adefovir dipivoxil; UV-Vis Spectrophotometry; Validation; Stability-indicating; Degradation

INTRODUCTION

Adefovir dipivoxil1 is chemically 9-[2-[Bis [(pivaloyloxy) methoxy] phosphinyl] methoxy]ethyl] adenine[1,2]. Adefovir dipivoxil is a diester prodrug of the active moiety adefovir, it is an acyclic nucleotide analogue of adenosine monophosphate[4]. It is a novel antiviral drug, which is highly efficient in the treatment of human hepatitis B virus (HBV) and HIV. It was reported that adefovir dipivoxil dose at 10 mg, once daily taken orally in the treatment of chronic hepatitis in adults with evidence of active viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or AST) or histologically active disease. Adefovir is phosphorylated to the active metabolite, adefovir diphosphate, by cellular kinases, adefovir diphosphate inhibits HBV DNA polymerases (reverse transcriptase) by competing with the natural substrate deoxyadenosine triphosphate and by causing DNA chain termination after its incorporation into viral DNA[5].

Literature survey reveals that Simple UV method [6], few bio-analytical methods were reported using human plasma by LCMS/MS,HPLC method [7,8,9] has been reported for determination of adefovir in bulk and pharmaceutical dosage forms. The literature survey also indicates that no stability indicating spectrophotometric method was proposed for adefovir . The aim of this work is to develop and validate an analytical method by using UV-VIS spectrophotometry for the estimation of adefovir in bulk and pharmaceutical dosage forms and also perform stress degradation studies on the drug as per ICH Guidelines using the developed method [10, 11].

MATERIALS AND METHODS

Materials

Reagents

a) Methanol (AR grade) (Merck)
b) HCl
c) NaOH
d) H_2O_2

Instruments

a) UV- visible spectrophotometer (2450 Shimadzu with UV probe 2.2.1 software), 10 mm quartz cell and spectral bandwidth 1nm

b) Micropipette, Variable volume 20 - 200 μL Biosystem classic

Determination of λmax

Preparation of Stock Solution

Standard stock solution of adefovir dipivoxil was prepared by dissolving 10mg of in adefovir dipivoxil 100ml of methanol to produce a concentration of 100μg/ml. which is the standard stock solution.

Preparation of standard stock solution and calibration curve

Standard stock solution containing 100 μg/ml of adefovir was prepared in methanol. Different aliquots were taken from the stock, diluted to 10 ml mark with same solvent to obtain series of concentrations. The solutions were scanned on spectrophotometer in the UV range 200 - 400 nm. Adefovir showed absorbance maxima at 260 nm **(Fig.1)**.
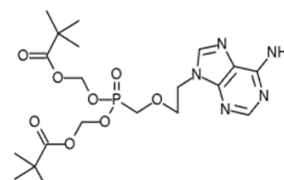

Fig. 1: Structure of Adefovir Dipivoxil

The optical characteristic and linear regression data is summarized in **Table 1.**

Table 1: Summary of optical characteristic and regression studies

Parameter	UV-Spectrophotometric
Wavelength	260 nm
Linearity equation (Y= a+ bc)	0.0259X+0.0069
Range (μg/mL)	5.00 - 40.00
Molar Absorptivity (lit/mol/cm)	7.5 X 10^3
Correlation Coefficient (r^2)	0.9999
S.D. of Slope	0.00014
S.D. of intercept	0.004053

Preparation of Sample Solution

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 10 mg of adefovir was transferred into 100 ml volumetric flask containing 30

ml methanol, shaken manually for 10 min., volume was adjusted to mark with same solvent and filtered through Whatmann filter paper no. 41. An appropriate aliquot was transferred to 10 ml volumetric flask, volume was adjusted to the mark and absorbance was recorded at 260 nm(**Fig.2**).
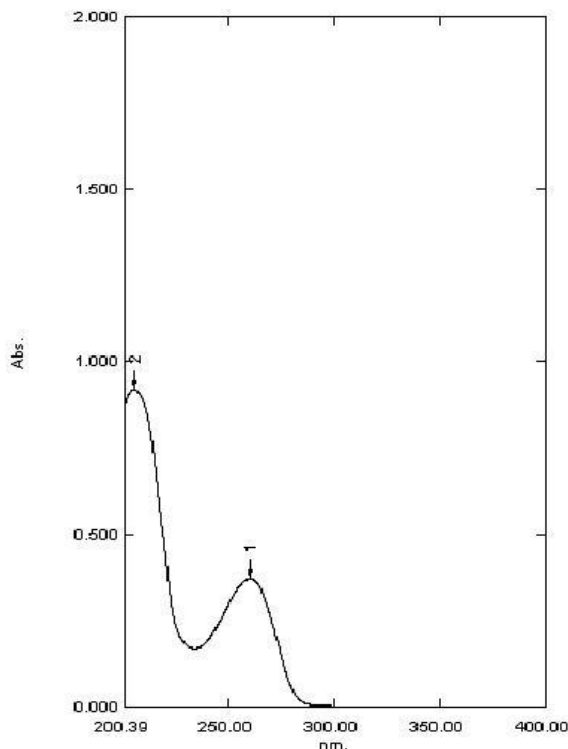

Fig. 2: λmax of Adefovir Dipivoxil showing at 260 nm

The concentration of the drug was calculated from linear regression equations; results are shown in **Table 2.**

Table 2: Results of Assay

Label claim	*Amount found (%)	
	UV-Spectrophotometric ±SD	%RSD
Adefovir Dipivoxil (10 mg/tablet)	99.88 ± 0.575	0.575

*mean of six determinations

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different

parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit Of Detection (LOD) and Limit Of Quantification (LOQ).

Linearity

Various aliquots were prepared form the stock solution (100μg/ml) ranging from 5- 40μg/ml. The samples were scanned in UV-VIS Spectrophotometer using methanol as blank. It was found that the selected drug shows linearity between the 5-40μg/ml

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which

the amount of marketed formulation(HESPERA -10mg) was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery

Precision

Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 9 different solutions of same concentration that is 20μg/ml were prepared and analysed three times in a day i.e. morning, afternoon and evening and the absorbance were noted. The result was indicated by % RSD. In the interday variation study, solutions of same concentration 20μg/ml were prepared and analysed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD

Specificity

10mg of Adefovir was spiked with 50% (5mg), 100% (10mg), and 150%(15mg) of excipient mix and the sample was analysed for % recovery of Adefovir.

Robustness

Robustness of the method was determined by carrying out the analysis at two different temperatures i.e. at room temperature and at 18^0c. The respective absorbance were noted and the result was indicated by % RSD.

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbance was noted. The result was indicated by % RSD **(Table 3).**

Table 3: Summary of validation parameters

Parameters	UV-Spectrophotometric
Linearity (µg/mL)	5.00 – 40.00
LOD	0.573
LOQ	1.736
% Recovery*	99.77%
(%RSD)	-0.574
Precision (%RSD)	
Intra-day (n = 3)	0.234 – 0.854
Inter-day (n = 3)	0.129 – 0.791
Repeatability(%RSD; n = 8)	0.565
Robustness(%RSD)	0.96
Specificity (%RSD)	1.26
Ruggedness (%RSD)	
Analyst I (% label claim)	100.26(0.229)
Analyst II(% label claim)	99.64(0.278)

*mean of nine estimations

Limit of Detection (LOD)

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

Limit of Quantification

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve.

Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Adefovir Dipivoxil using the method developed.

Stress degradation by hydrolysis under acidic condition:

To 2 ml of stock solution(1000µg/ml) of Adefovir Dipivoxil, 1 ml of 1 N HCl was added in 10 ml of volumetric flask and the volume was made up to the mark with methanol. Then, the volumetric flask was kept at normal condition for 90 minutes. After 60 min. time interval, 1 ml of solution was pipette out from this flask, neutralised and diluted with methanol in order to make the volume up to 10 ml and the dilution was carried out to achieve the appropriate concentration (20µg/ml). This solution was taken in cuvette. For the blank, 0.5 ml solution of 1N HCl and 0.5 ml solution of 1N NaOH were diluted with methanol in 10 ml of volumetric flask. After 90 minutes, again 1ml of the solution was pipetted out from the flask and the above procedure was repeated **(Fig.3, 4)**.

Stress degradation by hydrolysis under alkaline condition

To 2 ml of stock solution of Adefovir Dipivoxil 1 ml of 0.1 N NaOH was added in 10 ml of volumetric flask and made up the volume to the mark with methanol. Volumetric flask was kept at normal condition for 90 min. After 60 min time interval, 1 ml of solution was pipette out from this flask, neutralized and diluted with methanol in order to make the volume up to 10 ml and the dilutions were carried out to achieve the appropriate concentration (20µg/ml). The solution was then taken in cuvette. For the blank, 0.5 ml solution of 0.1N HCl and 0.5 ml solution of 0.1N NaOH diluted with methanol in 10 ml of volumetric flask. After, 90 minutes 1ml of solution was again pippetted out from the flask and the above procedure was repeated **(Fig.5,6)**.

Dry heat induced degradation:

Adefovir Dipivoxil sample was taken in a petri plate and exposed to a temperature of 50°c for 48 hours in an oven. After 48 hours, 10 mg of the sample was diluted with methanol in order to make the volume up to 10 ml. From this solution, dilutions were carried out to achieve the appropriate concentration (20µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis **(Fig. 7).**
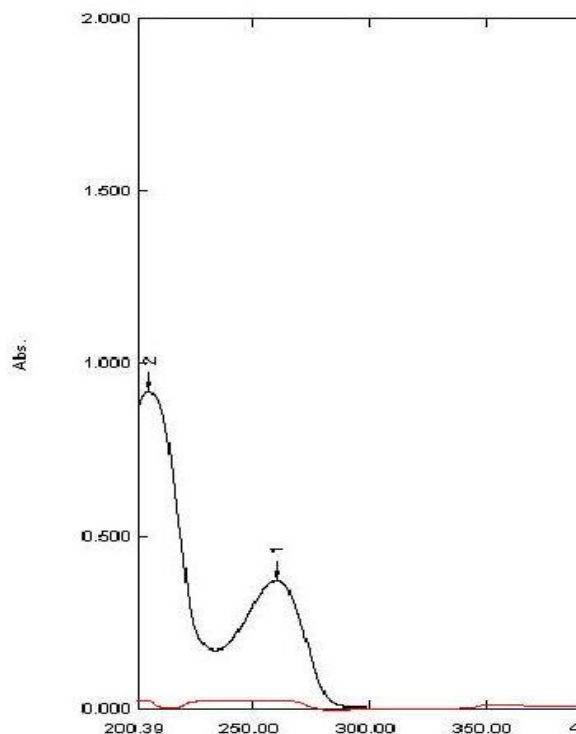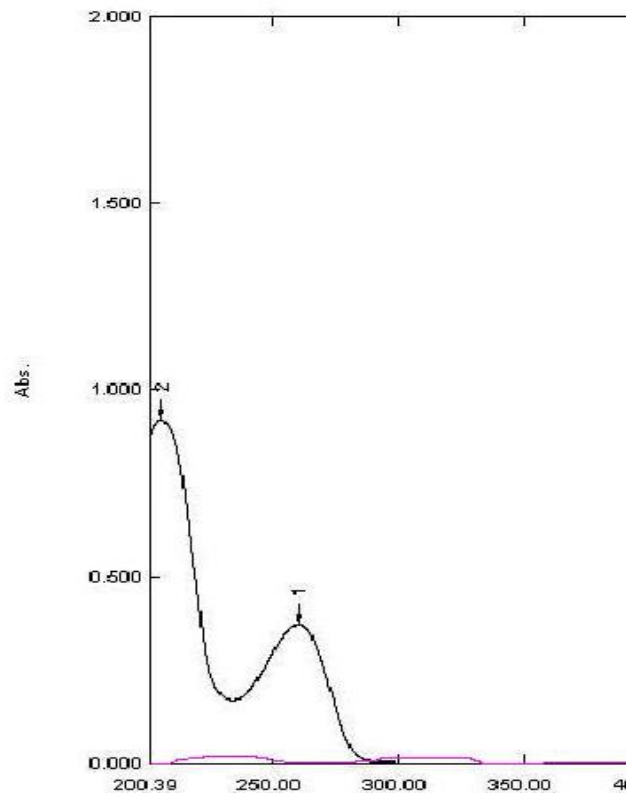

Fig. 3: Comparison between standard Adefovir Dipivoxil (20µg/ml) & Acid Degraded sample of Adefovir Dipivoxil (20µg/ml) after 60 minutes. Drug got degraded by 89.75% after exposing for 60min. to the acidic condition

Fig. 4: Comparison between standard Adefovir Dipivoxil (20µg/ml) & Acid degraded sample of Adefovir Dipivoxil (20µg/ml) for 90minutes. Drug got degraded by 98.79% after exposing for 90min. to the acidic condition
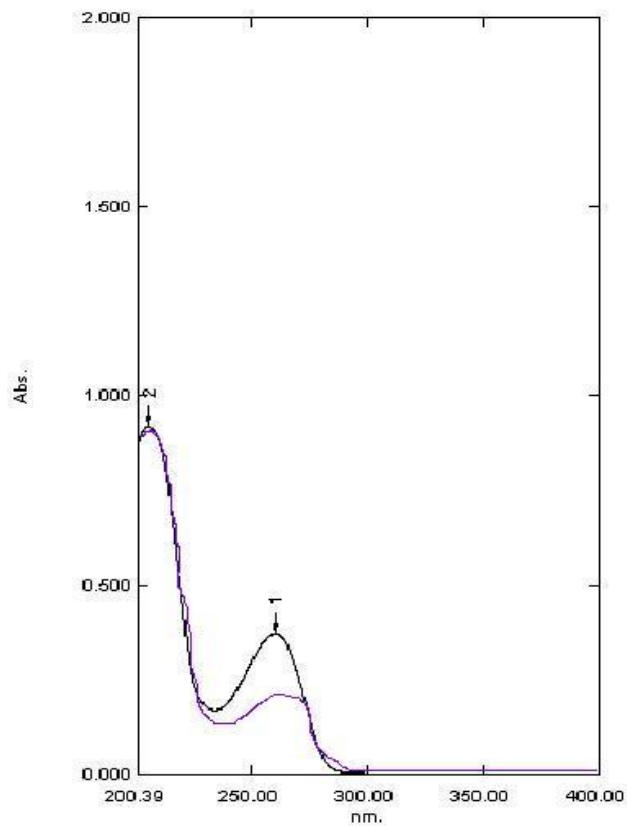

Fig. 5: Comparison between standard Adefovir Dipivoxil (20µg/ml) & Alkali degraded sample of Adefovir Dipivoxil (20µg/ml) after 60 minutes. Drug got degraded 43.07% after exposing for 60min. to the alkaline condition
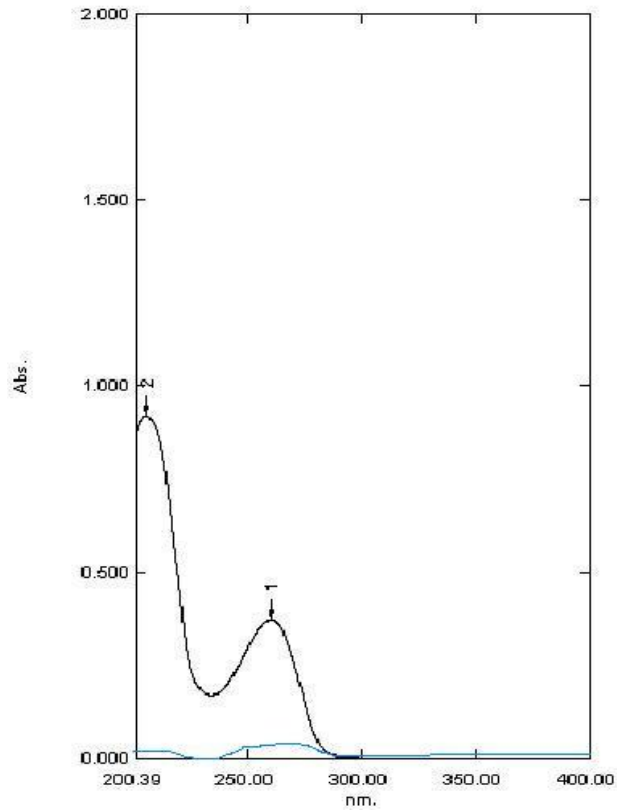
Fig. 6: Comparison between standard Adefovir Dipivoxil (20µg/ml) & Alkali degraded sample of Adefovir Dipivoxil after 90minutes.Drug got degraded by 87.95% after exposing for 90min. to the alkaline condition
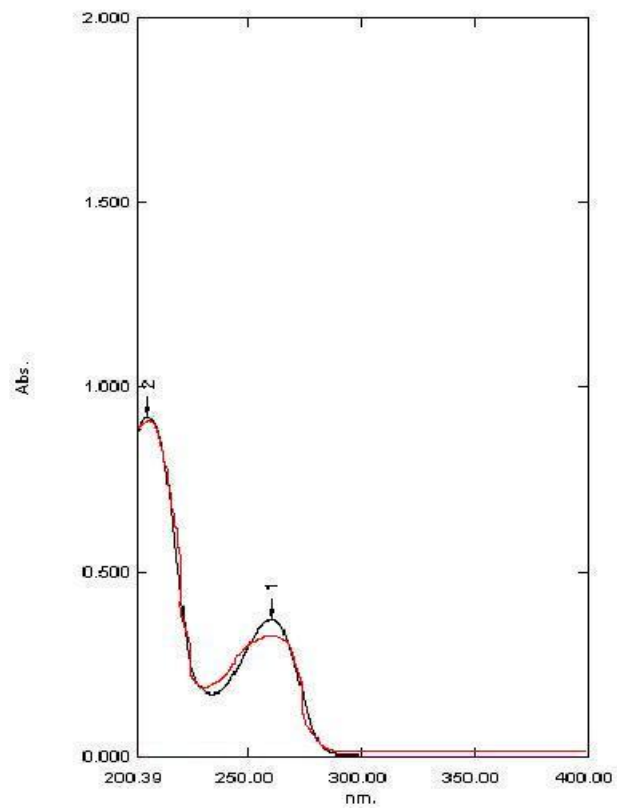

Fig. 7: Comparison between standard Adefovir Dipivoxil (20µg/ml) & Temperature degraded sample of Adefovir Dipivoxil (20µg/ml). Drug got degraded by 0.19% when exposed to a temp of 50°c for 48 hours.
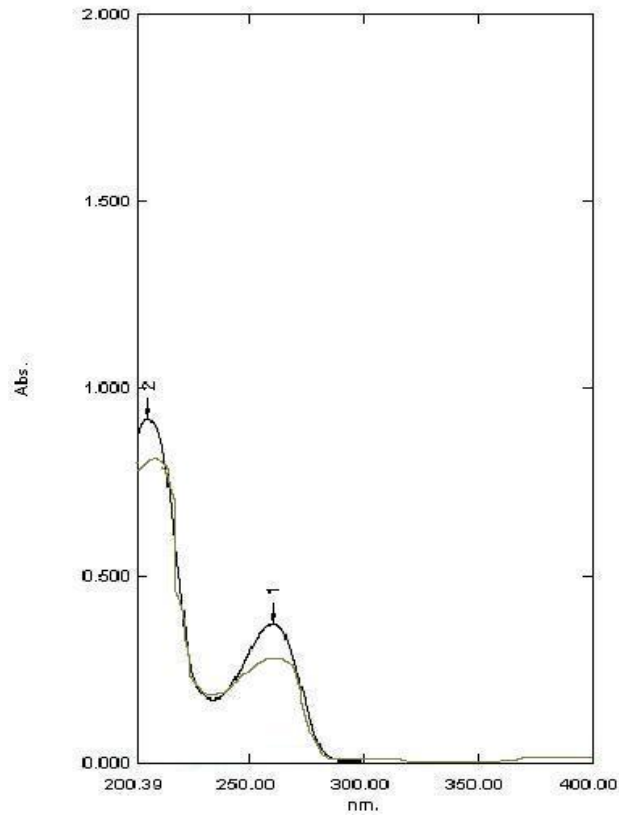
Fig. 8: Comparison between standard Adefovir Dipivoxil (20µg/ml) & Oxidized sample of Adefovir Dipivoxil (20µg/ml). Drug got degraded by 23.25% when it is treated with 30% (w/v) hydrogen peroxide for 15 minutes
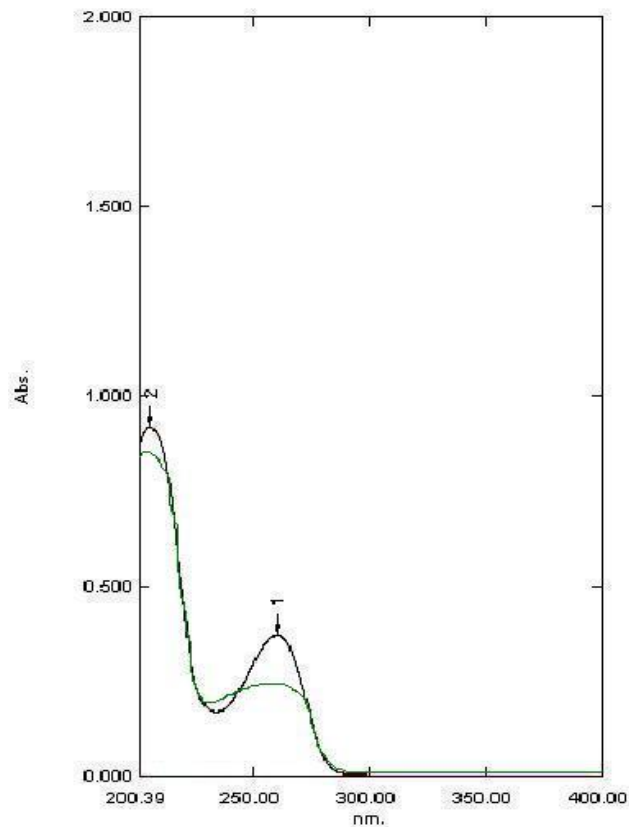

Fig. 9: Comparison between standard Adefovir Dipivoxil (20µg/ml) & UV degraded sample of Adefovir Dipivoxil (20µg/ml) after 3 hours Drug when exposed to UV light for 3hrs, got degraded by 34.53%

Oxidative degradation

To 1.5 ml of the stock solution of Adefovir Dipivoxil (1000µg/ml), 1 ml of 30 % w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with methanol. The volumetric flask was then kept at room temperature for 15 min. For the blank, 1 ml of the 30 % w/v of hydrogen peroxide was kept at normal condition for overnight in 10 ml of volumetric flask. Both solutions were heated on boiling water bath to remove the excess of hydrogen peroxide. Finally, after 15 minutes dilutions were made from the stock solution to achieve the required concentration (20µg/ml). The solution was then taken in a cuvette and analysed **(Fig. 8).**

Photolytic degradation

Sample of Adefovir Dipivoxil was exposed to near ultraviolet lamp in photostablity chamber providing illumination of not less than 1.2 million lux hours. Ten milligrams sample was dissolved methanol and volume made up to 10 ml. From this solution appropriate dilution (30µg/ml) was made using methanol and taken in cuvette for the U.V. analysis **(Fig. 9, 10).**

RESULT AND DISCUSSION

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (99.97% to 101.4%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 98.2% to 101.2%. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%. The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (99.77%). The stress degradation studies showed that Adefovir undergoes degradation in acidic and alkaline conditions whereas it is relatively stable when exposed to dry heat, oxidation and photolytic conditions.

Summary of the results of stress degradation studies of Adefovir are shown in the **(Table 4)**.

Table 4: Summary of result of stress degradation studies

Condition	Time	%Degradation
1N HCl(1ml)	60min	89.75%
	90min	98.79%
0.1N NaOH(1ml)	60min	43.07%
	90min	87.95%
30% Hydrogen Peroxide(1ml)	15min	23.25%
Dry Heat 50°	48hr	0.19%
Photolytic	3hr	34.53%
	6hr	41.36%

CONCLUSION

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of adefovir in bulk and pharmaceutical formulation. The proposed method is also useful for determination of adefovir stability in sample of pharmaceutical dosage forms.

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