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SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF ADEFOVIR DIPIVOXIL IN BULK AND TABLETS

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ABSTRACT

Two simple, rapid, sensitive and accurate UV- Spectrophotometric and first order derivative methods have been developed for estimation of Adefovir in bulk and tablets. In methanol: water (3 :7), the λ_{max} of Adefovir was found to be 260 nm and the same spectrum was derivatized into first order derivative using UV probe software of instrument (shimadzu 2450), at $\Delta\lambda$ = 2. The amplitude of the trough was recorded at 273 nm. In both these methods, linearity was observed in the concentration range of 4 – 32 µg/mL. The assay results of both these methods were in good agreement with label claim. The methods were validated statistically and recovery studies.

KEYWORDS: **Adefovir, UV-Spectrophotometric method, first order derivati spectrophotometry**

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INTRODUCTION

Adefovir dipivoxil1 is chemically 9-[2-[Bis [(pivaloyloxy) methoxy] phosphinyl] methoxy]ethyl] adenine[1,2,3]. 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[5] (reverse transcriptase) by competing with the natural substrate deoxyadenosine triphosphate and by causing DNA chain termination after its incorporation into viral DNA[6]. Literature survey reveals that Simple UV method [7], few bio-analytical methods were reported using human plasma by LCMS/MS[8,9],HPLC method [10] 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 for the estimation of adefovir in bulk and pharmaceutical dosage forms by using UV-VIS spectrophotometry and first order derivative spectrophotometry [11]

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MATERIALS AND METHODS

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

Reagents

a) Methanol (AR grade) (Merck)
b) Double Reverse Osmosis (R.O.) water

(i) Procedure

Standard stock solution containing 100 µg/ml of Adefovir was prepared in methanol:water(3:7). From the stock solution different aliquots of 0.4,0.8,1.2,1.6,2.0,2.4,2.8,3.2 ml were taken in volumetric flask and, diluted upto 10 ml with water to obtain a concentrations of 4,8,12,16,20,24,28,32 ug/ml . The solutions were scanned on UV spectrophotometer in the range of 200 - 400 nm. Adefovir showed absorbance maxima at 260 nm(Fig.1). The spectra obtained were derivatised into first order derivative, using UV probe software of instrument, where $\Delta\lambda = 2$ (Fig.2).Adefovir had the zero crossing at 273nm The amplitudes of the corresponding troughs were measured Calibration curve were plotted in between amplitudes observed at first order against the concentration in the range of of 4-32µg/ml. The optical characteristic and linear regression data is summarized in Table 1

Figure 1
UV- spectrum of Adefovir in methanol:water (3:7)

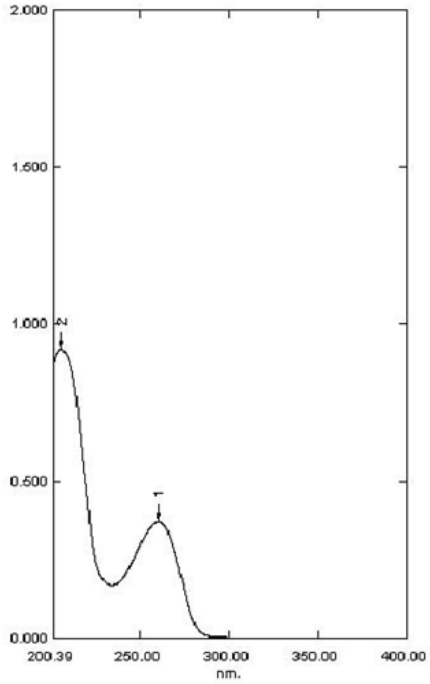

Figure 2
An Overlain first order derivative spectra of Adefovir in methanol:water(3:7)

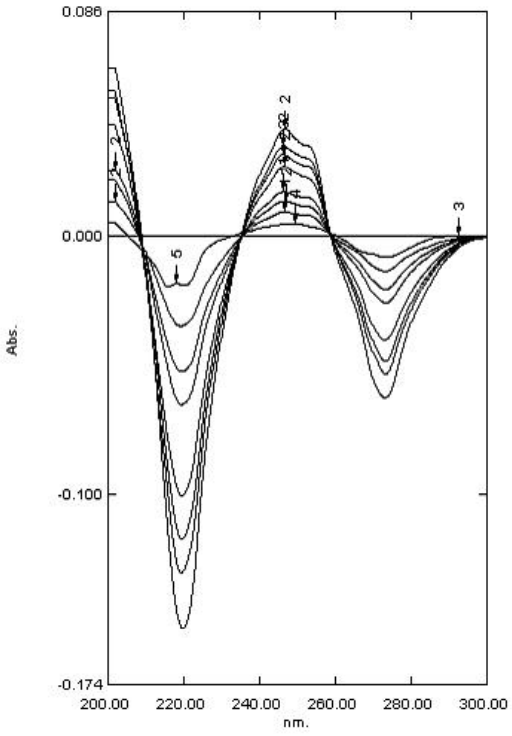

Table 1
Summary of optical characteristic and regression studies

Parameter	UV-Spectrophotometric	First order derivative
Wavelength	260 nm	273 nm
Linearity equation (Y= a+ bc)	0.0259X+0.0069	0.00132X+0.00039
Range (µg/mL)	4.00 - 32.00	4.00 – 32.00
Molar Absorptivity (lit/mol/cm)	7.5×10^3	-------
Correlation Coefficient (r^2)	0.9999	0.9997
S.D. of Slope	0.00014	0.000024
S.D. of intercept	0.004053	0.00187

(ii) 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 water 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. The same spectrum was derivatised using UV-probe software and amplitude of the trough was recorded at 273 nm. The concentrations of the drug was calculated from linear regression equations; results are shown in

Table 2
Table II – Results of Assay

Label claim	*Amount found (%)			
	UV-Spectrophotometric ±SD	%RSD	First order derivative ±SD	%RSD
Adefovir Dipivoxil (10 mg/tablet)	99.88 ± 0.575	0.575	99.78 ±0.185	0.277

mean of six determinations

RESULTS AND DISCUSSION

Adefovir in methanol:water(3:7) showed absorbance maximum at 260 nm. In first order derivative, the amplitude of the trough was recorded at 273 nm. In both the methods, Adefovir follows linearity in the concentration range of 4 - 32 µg/ml. In both these methods, amount of drug determined was in the good agreement with the label claim. The methods were validated[12] for linearity, accuracy, precision, ruggedness and robustness. The accuracy of the methods was assessed by recovery studies at three different levels i.e. at 80%, 100% and 120%. In both the methods, %RSD values were found to be less than 2, indicative of accuracy of the method. The precision of the methods were studied as intra-day, inter-day and repeatability. The % RSD values less than 2 indicate the methods are precise. Ruggedness of the proposed method was studied with the help of two analysts.; the % RSD value less than 2 indicate methods are rugged. The results from validation studies are shown in Table 3.

Table 3
Summary of validation parameters

Parameters	UV-Spectrophotometric	First order derivative
Linearity (µg/mL)	4.00 – 32.00	4.00 - 32.00
LOD	0.573	0.641
LOQ	1.736	1.943
% Recovery* (%RSD)	99.77 % (0.574)	100.04 % (0.314)
Precision (%RSD)		
Intra-day (n = 3)	0.234 – 0.854	0.266-1.257
Inter-day (n = 3)	0.129 – 0.791	0.362 - 1.161
Repeatability(%RSD; n = 8)	0.565	0.302
Ruggedness (%RSD)		
Analyst I (% label claim)	100.26 (0.229)	99.75 (0.167)
Analyst II(% label claim)	99.64 (0.278)	100.08 (0.465)

mean of nine estimations

CONCLUSION

Both these methods are simple, rapid and accurate and precise and can be used for routine analysis of Adefovir from tablet formulations

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