Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(1):128-133

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Nucleophilic substitution method for the determination of Adefovir in bulk and formulations

Murthy. Ch. V. R[1], Acharyulu. M. L. N[*2], Reddy. T. S[3] and Srinivas. B. V[4]

[1]Department of Chemical Engineering, AU College of Engineering (A), Andhra University, Visakhapatnam, Andhra Pradesh, India
[2]Department of AS&H, VITAM College of Engineering, Mindivanipalem Anandapuram Mandal,Visakhapatnam, A. P., India
[3]DLR College (Degree & P.G. Courses), Gollalamamidada, E.G.Dtt, A.P.
[4]Department of Organic Chemistry, Foods, Drugs and Water, A.U, Visakapatnam

---

ABSTRACT

A Simple and rapid spectrophotometric method has been developed and validated for the determination of Adefovir (ADE) bulk and formulations. The proposed method is based on nucleophilic substitution reaction of ADE with 1,2-naphthoquinone-4-sulphonate (NQS) in an alkaline medium to form an orange-colored product of maximum absorption peak (λmax) at 450 nm. The stoichiometry and kinetics of the reaction were studied, and the reaction mechanism was postulated. Under the optimized reaction conditions, Beer's law correlating the absorbance with ADE concentration was obeyed in the range of 1–7µg mL^{-1}. The limits of detection and quantitation were 0.3 and 0.8µg/ mL^{-1} respectively. The proposed method was successfully applied to the determination of ADE in its pharmaceutical tablets with good accuracy and precisions; the label claim percentage was 99.56 ± 0.45 to 99.92 ± 0.20.

Key Words: spectrophotometriy, 1,2-naphthoquinone-4-sulphonate (NQS),Alkaline medium

---

INTRODUCTION

The chemical name of Adefovir dipivoxil is 9-[2-[bis[pivaloyloxy) methoxy]phosphinyl] methoxy]ethyl]adenine. Adefovir is an acyclic nucleotide analog with activity against human hepatitis B virus (HBV). It is an acyclic nucleotide analog with activity against human HBV.It is important to develop and validate analytical method for its determination in pharmaceutical dosage forms. The method was validated by parameters such as correlation coefficient, intercept and slope. A very few physio-chemical methods appeared in the literature for the determination of ADE[1-5] in pharmaceutical formulations (less) the methods so far reported includes TLC, spectrophotometric (UV and visible), Tandem mass spectrometry.The analytically important functional groups of ADE are active methylene, purine, primary amine, phosphate ester were not properly exploited designing suitable spectrophotometric methods for the determination of the selected drug.The reaction of 1,2-napthaquinone -4-sulfonic acid (NQS) with primary aromatic amines was discovered by Boniger[6]as far back as 1894.The applications of NQS for spectrophotometric methods [7-18] as the chromogenic reagent are in good number and it is simplest reagent because of its well stability and the stability of colored complexes it formed with a series of reagents was been well utilized by good number of researchers. It is clear from the literature that usage of NQS for

the determination of ADE, the selected drug by the author was not attempted.Therefore in this paper, the author has made a valid attempt to develop a sensitive and reproducible method for the assay of the ADE. The author has also developed simple and sensitive UV method (0.1N HCl as solvent) and adopted it as a reference method(**Fig**s.**4,5)** for comparing accuracy of the results obtained by the proposed method for ADE.The present paper describes the evaluation of NQS as a chromogenic reagent in the development of simple and rapid spectrophotometric method for the determination of ADE in its tablets.It is official in [19-20]

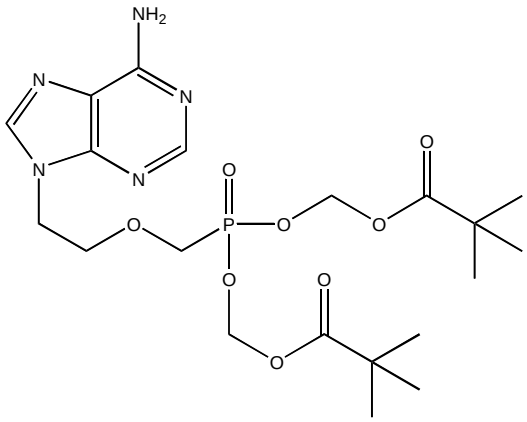

Fig.1.Chemical Structure of ADE

EXPERIMENTAL SECTION

Chemicals and reagents

All the chemicals and reagents were of analytical grade and the solutions were prepared freshly.

NQS solution(Luba, 0.075%,2.38x10^{-3}M) is prepared by dissolving 75mg of NQS in 100ml of distilled water, NaOH solution(E.Merck,10%,2.5M) is prepared by dissolving 10 g of NaOH in 100ml of distilled water.

Apparatus

A UV –1601 and SHIMADZU digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. A SYSTRONICS digital pH meter 361 was used for pH measurements.

Preparation of standard solutions

A 1mg/mL solution was prepared by dissolving 100mg of pure ADE in 100ml of water and further diluted to 400 µg/mL

Procedure: Aliquots of standard drug solution (0.25ml – 2.5ml) 400µg/mL were placed in a series of 20ml calibrated test tubes. Then 2.5 ml of NQS and 2.0mL of NaOH solution were added to each tube and kept aside at room temperature. The solution was made up to the mark with distilled water. The absorption was measured at 450nm against reagent blank prepared simultaneously (**Fig.2**). The amount of ADE was deduced from the Beer's plot (**Fig.3**).

Preparation of formulation samples

The tablet powder equivalent to 100 mg of ADE was extracted with 3x25 mL of chloroform and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in 100ml of distilled water to achieve a concentration of 1mg/mL stock solution.The solution was further diluted step wise with distilled water to get working standard solutions and analysed under procedures described for bulk samples.

RESULTS AND DISCUSSION

The optimum conditions for this method were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the coloured species. Beer's law limits, molar extinction coefficient, Sandell's sensitivity and regression characteristics of the method are presented in Table-1. The

relative standard deviation and % range of error are also given in Table-1. Recovery studies were carried out by addition of known standard drug solution to pre analyzed sample solution. Results of recovery studies were presented in Table-2.The interference studies in the determination of ADE in pharmaceutical formulations revealed that the normally existing excipients and additives like hydroxyl propyl cellulose, lactose, carboxy methyl cellulose were found not to interfere even when present in excess. In developing the method, a systematic study of the effects of various relevant parameters in the concerned were under taken by varying one parameter at a time and controlling all other parameters to get maximum colour development, minimum blank colour, reproducibility and reasonable period of stability of final coured species formed.

Nature of coloured species

The reaction of 1,2–napthaquinone–4-sulfonic acid (NQS) with primary aromatic armines was discovered by Boniger as far back as 1894.The colored species formed from ADE in this method can be explained based on the analogy of previous reports [21-22]. As ADE possesses primary amino groups over purine moiety it involves in yielding coloured produced by nucleophillic displacement of the Sulfonic acid group of 1,2-naphthaquinone-4-sulfonic acid in alkaline conditions.

TABLE:1 OPTICAL CHARACTERISTICS, PRECISION, ACCURACY OF THE METHOD PROPOSED IN THE DETERMINATION OF ADE

S.No	OPTICAL CHARACTERISTICS	NQS
1	λ_{max}(nm)	450
2	Beer's Law limits(µg/ml)	1-7
3	Molarabsorptivity(l $mol^{-1}cm^{-1}$)	$4.03x10^{4}$
4	Correlation coefficient (r)	0.9999
5	Sandell'ssensitivity (µg/cm^2/0.001absorbance unit)	0.001
6	Regression equation(y=a+bc) (i)slope (b)	0.0455
	(ii) Standard deviation on intercept(S_b)	$1.96x10^{-4}$
	(iii)intercept (a)	0.0029
	(iv) standard deviation (Sa)	$7.66x10^{-4}$
	(v)Standard error of estimation(S_e)	$8.23x10^{-4}$
7	Optimum photometric range (µg/ml)	6.99-12.99
8	Relative Standard deviation *	0.7044
9	Detection limit	0.00505
10	% of range of error(confidence limit) (i)0.05 level	0.7347
	(ii)0.01 level	1.209

Table-2.DETERMINATIONOF ADE IN PHARMACEUTICAL FORMULATIONS

SAMPLE	LABELLED AMOUNT(mg)	AMOUNT FOUND	
		PROPOSED METHOD	REFERENCE METHOD
Tablets– T_1	200mg	99.96 ± 0.283 t = 1.09 F = 1.96	99.57 ± 0.25
Tablets– T_2	200mg	99.73 ± 0.460 t = 0.51 F = 2.15	99.92 ± 0.20
Tablets– T_3	200mg	100.04 ± 0.462 t = 1.00 F = 1.32	99.56 ± 0.45
Tablets– T_4	200mg	99.84± 0.475 t = 0.94 F = 1.45	99.83 ± 0.50

**Tablets from four different pharmaceutical companies.*
***Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95 % confidence limit,F=5.05,t=2.57.*

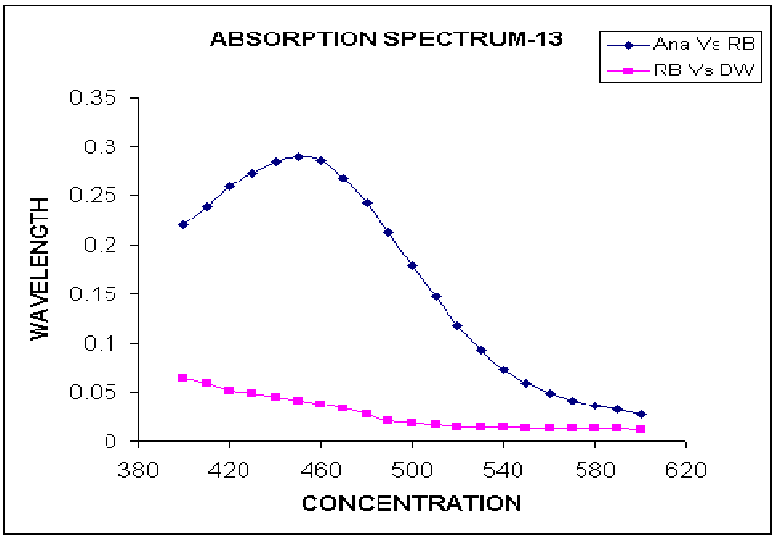

Fig.2 Absorption Spectrum of ADE with NQS/NaOH

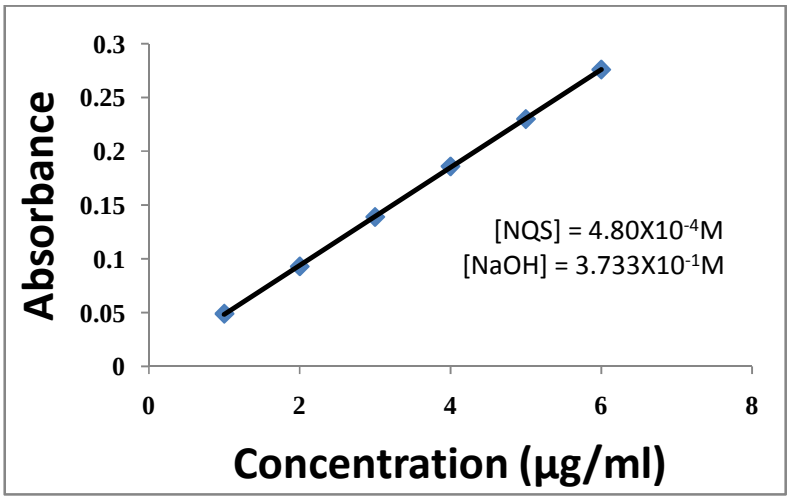

Fig.3.Beer"s Plot of ADE withNQS/NaOH

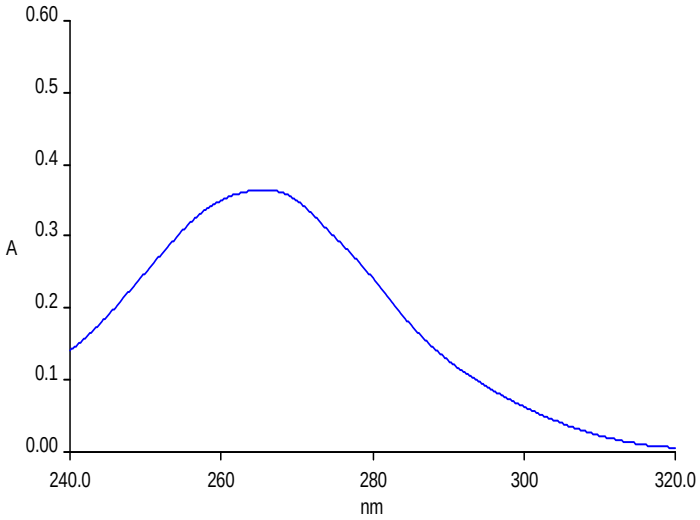

Fig.4.UV spectrum of 10 µg/ml solution of ADE in 0.1 N HCl
Wavelength max = 265 nm, Absorbance = 0.369
Slope = 0.0364, Intercept = 0.0003, CC = 0.9998

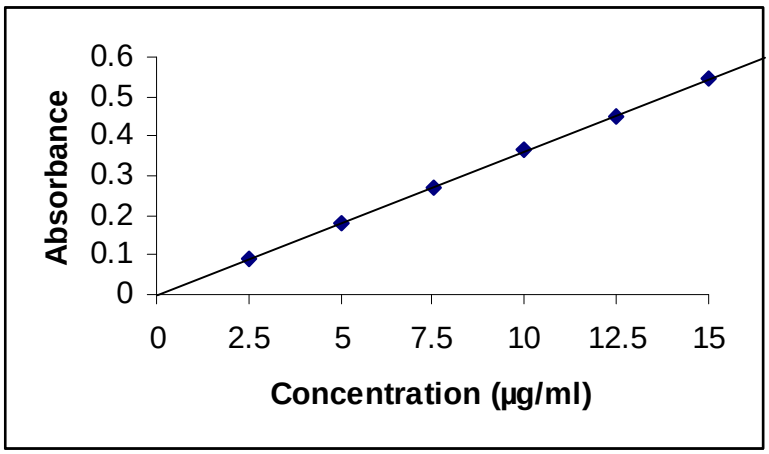

Fig.5. Beer's Plot of ADE (UV Reference Method)

CONCLUSION

The proposed method is superior in one way or other in terms of simplicity,λ_{max},ϵ_{max} stability of coloured species over very few visible spectrophotometric methods reported so far. It can be seen from the results presented above, that the proposed method has good sensitivity and λ_{max}. Stastical analysis of the results (Table 1) shows that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for the analysis with virtually no interference of the usual additives. The proposed method is simple, sensitive, and reliable and can be used for routine determination of ADE in bulk samples and pharmaceutical formulations depending upon the needs of the specific situation.

Acknowledgements
The author* express his deep sense of gratitude to UGC, SAP, PHASE-III for its assistance.

REFERENCES

[1].Hosseini,S.Y,Sababi,F,Samini-Bavil-Olyaee.SM,*Journal of Virological Methods*, **2006.**
[2]. Sanchez-Moreno, M., Gomez-Coca, R. B., Fernandez, A., *Journal of Biomol. Chem*, **2003.**

[3]. Parra, D.,Gonzalez,A,Muguetta.C,Martinez.A, *Journal Physiol. Biochem* **2001.**
[4].Ueo.H, Motohashi.H,Kataura.T,Klnui, *Biochem Pharmacols*,**2005**.
[5]. Murakami, E., Bao, H., Ramesh, M., Mc Bratee, J. R., *Journal of Antimicrobial Agents and Chromatography*, **2007**.
[6]. Boniger,M.,**1894**,27,23.
[7].Ibrahim A. Darwish, Heba H. Abdine, Sawsan M. Amer, Lama I.Al-Rayes, *International Journal of Analytical Chemistry*,**2009**; Article ID 237601, 8 pages,doi:10.1155/2009/237601
[8].Ibrahim.A,Darwish**,**Mona,M.Al-Shehri,Manal,A.El-Gendy**,***ChemistryCentralJournal*,**2012,** 6,11
[9].Aswani Kumar. Ch, Gurupadayya. B. M, Navya Sloka. S, Chandan. R S, Thejaswini. JC, *Tropical Journal of Pharmaceutical Research*, **2011,**10,81-88
[10].Ch.Aswani Kumar, T. Anil Kumar, B. M. Gurupadayya, S. Navya Sloka,M.B.Rahul Reddy, *Scholars Research Library, Archives of Applied Science Research*, **2010**,2,278-287
[11]. Ashraf M. Mahmoud, Nasr Y. Khalil, Ibrahim A. Darwish, and Tarek Aboul-Fadl , *International Journal of Analytical Chemistry*,**2009**, Article ID 810104
[12].Sara.A.M, Ebraheem,Abdalla.A, Elbashir, Hassan.Y,Aboul-Enein,*Acta Pharmaceutica Sinica B*, **2011**,248–253.
[13]. Spectrophotometric determination of pyrimethamine (PYM) in pharmaceutical formulation using 1,2-naphthoquinone-4-sulfonate (NQS), *Journal of the Association of Arab Universities for Basic and Applied Sciences*, **2012,**11,32–36.
[14].Q.M.Li,J. Li, and Z.-J. Yang, 152, 2007.
[15].M. Hasani, L. Yaghoubi, and H. Abdollahi, *Analytical Biochemistry*, **2007,**365,74–81.
[16].Q.M.Li,Z.-J.Yang, *Spectrochimica Acta Part A*, **2007**,66,656–661
[17].I.A.Darwish, *Analytica Chimica Acta*, **2005,**551,222–231,
[18].L. Xu,H.Wang,Y.Xiao, *Spectrochimica Acta Part A*, **2004**,60,3007–3012,
[19]. The Merck Index, 13th Edn. Merck & Co Inc, New York, **1996**,29.
[20]. Physicien Desk Reference (PDR) 60th Edn, **2006**,1275
[21]. Ismiel, S. A., Yassa, D. A., Attia, H. A., *Pharazie*, **1974**,29,348.
[22]. Kolsel, J., Perpar, M., *J. Anal.chem.*, **1959**,167,161