International Journal of Engineering, Science and Mathematics
Vol. 9 Issue 2, February2020,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

VALIDATION OF DARUNAVIR IN PHARMACEUTICAL FORMULATIONS USING MBTH REAGENT

BY

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ABSTRACT

A simple and sensitive extractive visible spectrophotometric method for the assay of Darunavir (DNV) in pure and pharmaceutical formulations is developed., The method is based on oxidizable centre of primary amine the of selected drug reacts with MBTH reagent in presence of Ce (IV) to give coloured species at absorption peak of λmax at 518 nm. Beer's law is obeyed in the concentration range 4-24 µg/ml, the Molar absorptivity and Sandell sensitivity are 1.8295 $x10^5$ and 1.3540 $x10^{-3}$ µg cm^{-2} respectively. The method proposed gave reproducible results with the percentage recoveries in the formulations found to be 99.800 to 99.949.The proposed method is cheap, accurate and can be successfully applied for the determination of DNV.

KEYWORDS: Spectrophotometry; MBTH; Cerium(IV),Anti retroviral,Pharmaceutical formulations.

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INTRODUCTION

Darunavir (DNV), (Fig.1), is an oral anti-retroviral agent which selectively inhibits the cleavage of Human immunodeficiency virus (HIV-1) encoded Gas-polyproteins in infected cell, thereby preventing the formation of mature virus. Darunavir ethanolate is chemically [(1S,2R)-3-[[(4-amino phenol) sulfonyl](2-methyl propyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl ester monoethanolate1. This drug is effective in patients experienced in anti-retroviral treatment, such as those carrying HIV-1 strains which are resistance to more than oPI[2].The use of

advanced instrumentation techniques for the analysis of drugs has been discussed elsewhere[3]. Literature survey revealed that different analytical methods have been reported for the determination of DNV in plasma using liquid chromatography coupled with tandem mass spectroscopy[4] simultaneous determination of DNV with other anti-retroviral agents in plasma[5,6]. Few HPTLC methods for determination of DNV in rat plasma and in tablet dosage form its application to pharmacokinetics studies[7]. Infrared Spectro scopy method for determination of Darunavir in tablets[8]. Few methods had been developed for determination of DNV by HPLC[9-13]and electrophoretic method for the separation of DNV[14]and Spectrophotometric method[15-22]

The analytical useful functional groups in DNV have not been fully exploited for designing suitable visible spectrophotometric methods and so still offer a scope to develop more visible spectrophotometric methods with better sensitivity, precision and accuracy. The author has made some attempts in this direction and succeeded in developing six methods in part-A. All these methods have extended pharmaceutical formulations as well. Literature survey reveals that reported HPLC methods require more time for sample analysis resulting in lesser throughput. Krishna Kumar Rao et al have estimated Darunavir Ethanolate by spectrophotometry[23] Therefore the author has made an attempt to develop rapid RP-HPLC method for determination and estimation of DNV in bulk and tablet dosage form. Validation as per USFDA and ICH guidelines [24,25].Upon thorugh literature survey done by the author,it is clear that no attempt has been made by earlier authors to make use of useful functional groups in DNV for its determination by visible spectrophotometric methods

EXPERIMENTAL

Instruments Used

A Schimadzu UV-Visible spectrophotometer 1801 with 1 cm matched quartz cells was used for all spectral and absorbance measurements. A Systronics digital pH meter 361 was used for pH measurements.

Preparation of standard Drug solution

The stock solution (1mg/ml) of Darunavir (DNV) was prepared by dissolving 100 mg of it in 100 ml of millipore-distilled water. A portion of this stock solution was diluted stepwise with the distilled water to obtain the working standard DNV solution of concentrations 30µg/ml for the proposed method

Procedure of Assay of DNV in formulations

An accurately weighed amount of formulation (tablet) equivalent to 100 mg of drug was dissolved in 20 ml of distilled water, shaken well and filtered. The filtrate was further diluted to 100 ml with distilled water to get 1 mg/ml solution of drug in formulations. One ml of this solution was furthered diluted to 25 ml to get 40 µg/ml solution. The absorbance of the solution was determined λ_{max} 223 nm (Fig.2). The quantity of the drug was computed from the Beer's law plot (Fig.3) of the standard drug in distilled water.

Recommended Procedure:

After systematic and detailed study of the various parameters involved, as described under results and discussion in this chapter, the following procedures were recommended for the determination of DNV in bulk samples.

To 20 ml graduated test tubes containing list volumes (0.1-0.6 ml) of 24 λ_{max}/ml DNV solution, 1.5 ml of MBTH solution were added. The resulting solution allowed to stand for 2 min at room temperature. After that 1.5 ml of Ceric ammonium sulphate solution was added, then allowed to react 5 minutes and diluted to 20 ml with distilled water. Absorbance was measured during the next 20 min at λ_{max}518 nm(Fig.4) against reagent blank prepared in a similar manner omitting the drug. The amount of DNV in a sample was computed from Beer- Lambert's law,(Fig.5).

RESULTS AND DISCUSSION

Spectral characteristics

In order to ascertain the optimum wave length of maximum absorption of the colored species formed in each of the above method, specified amounts of DNV in the final dilution 30µg/ml for proposed method were taken and the colours were developed separately following the above mentioned procedures. The absorption spectra were scanned in the wavelength region of 360-850 nm against corresponding reagent blank. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results are graphically presented in the Fig.4 and in Table.1.Also the proposed procedure is applied to pharmaceutical samplesand the obtained sprectral chracteristics were given in Table.2

Chemistry of coloured species in the present investigation

DNV possesses different functional moieties such as primary amine, tertiary amine and sulphonyl groups of varied reactivity. The proposed method is based on reactivity of primary amine and the colour development with MBTH/Ce(IV).In the present investigation MBTH in presence of ce(IV) combination has been used for the determination of DNV, as the selected drug oxidizable centre which reacts with MBTH reagent in presence of Ce(IV) to give coloured species which is valuable for the determination of drug by visible spectroscopy. The reactions are described in the scheme in Fig.6

CONCLUSION

The results presented above indicate that the proposed method has good sensitivity, selectivity, precison and accuracy. Results of analysis of bulk form and formulations reveal that the proposed method is suitable for the estimation of DNV, as impurities and excipients present in them cause no interference virtually.

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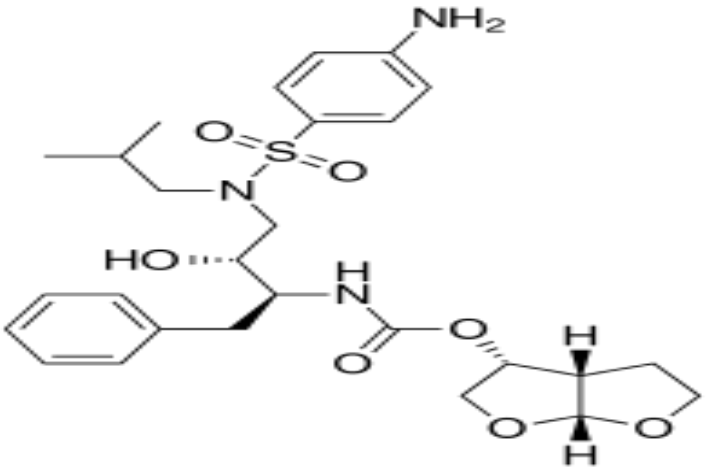

Fig.1

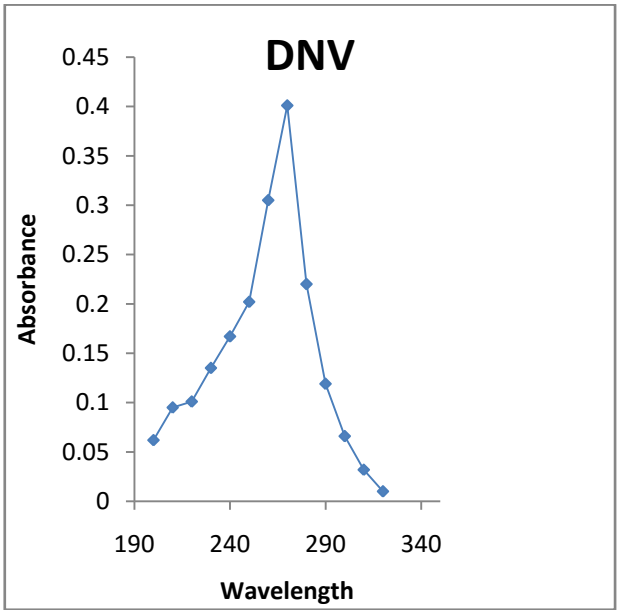

Fig.2 Absorption spectra of DNV in methanol (UV reference method)

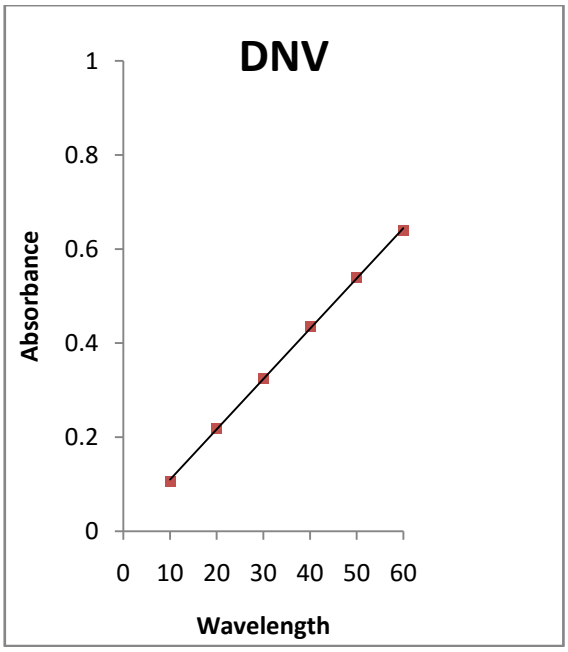

Fig. 3 Beer's law Plot of DNV in methanol
(UV reference method)

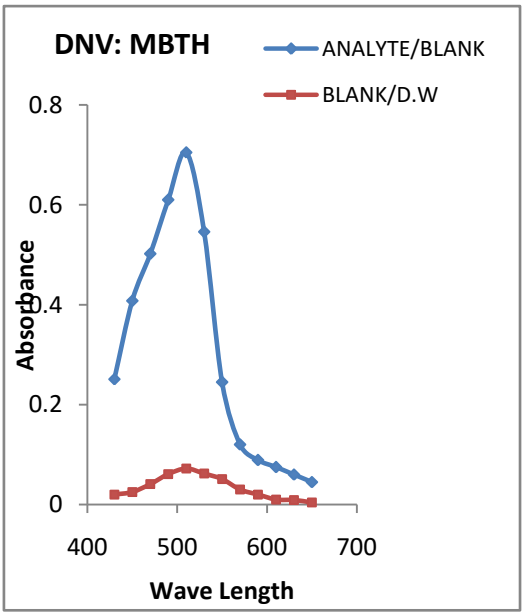

Fig .4 Absorption spectra of DNV: MBTH

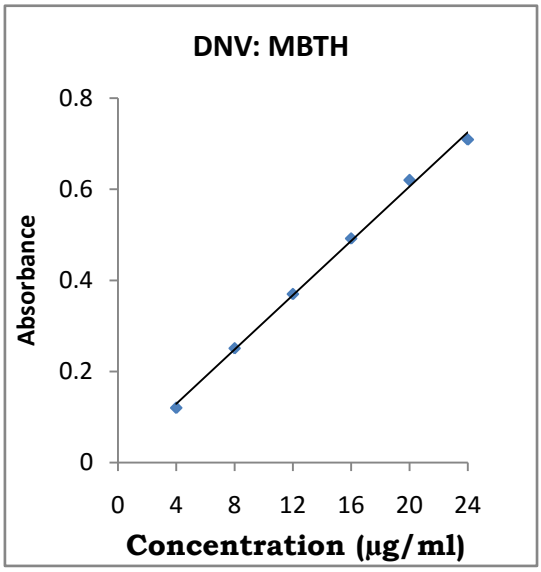

Fig. 5 Beer's plot of DNV: MBTH

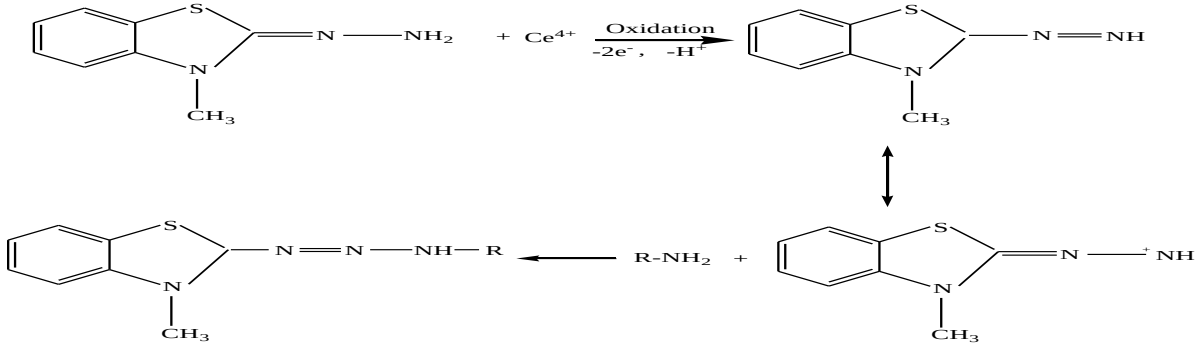

Fig. 6

S.No	Parameter	Values
1	Wave length λ_{max} (nm)	518
2	Beer's law limits (µg ml^{-1})	4-24
3	Detection limits (µg ml^{-1})	1.1314
4	Molar absorptivity (1 mole cm^{-1})	1.8295 x10^{5}
5	Sandell's sensitivity (µg cm^{-2} / 0.001 absorbance unit)	1.3540 x10^{-3}
6	Regressionequation (Y = a + bC) Slope (b)	0.0298
7	Standard deviation of slope (S_b)	7.2148 x10^{-4}
8	Intercept (a)	0.0096
9	Standard deviation of intercept (S_a)	1.1239x 10^{-2}
10	Standard error of estimation (S_e)	1.2070 x 10^{-2}
11	Correlation coefficient (r^2)	0.9977
12	Relative standard deviation (%)*	0.7065
13	% Range of error0.05 level	0.7415
14	% Range of error 0.01 level	1.1630
15	% Error in bulk samples/ % recovery	0.176

Table.1 Optical, Regression characteristics, precision and accuracy of the proposed methods for DNV

*: Average of six determinations considered

S.No	Sample	Amount taken (mg)	Amount found by proposed method	Amount found by reference method	Percentage recovery by proposed method
1	Tablet I	300	299.0±0.162 F=1.316 t=1.35	299.3±0.186	99.800±0.284
2	Tablet II	300	299.07±0.391 F=1.052 t=1.42	299.25±0.401	99.949±0.168

Table.2 Assay and recovery of DNV in Pharmaceutical Formulations

*: Average ± standard deviation of six determinations; the t- and F- values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit t=2.57, F=5.05.