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Spectrophotometric determination of Darunavir using NQS and Brucine meta periodate

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ABSTRACT

Two simple and sensitive spectrophotometric methods (A and B) for the assay of Darunavir (DNV), in pure and pharmaceutical formulations were developed. The method-A is based on the condensation reaction of DNV forming an orange-red colored Schiff's base of maximum absorption peak (λ max) at 444 nm with sodium 1,2-naphthaquinone-4-sulfonate (NQS) in an alkaline medium. The dimethoxy benzene nucleus of brucine is attacked by IO4- with the formation of O-quinone (Bruci quinone) which in turn undergoes nucleophilic attack on the most electron rich position of the coupler i.e., proton bearing amino group (primary amine of DNV), to give 1-mono substituted Bruci quinone derivative. The reaction mechanism in both methods were discussed. The absorbance of colored complex is found at 511nm. Beer's law is obeyed in the concentration range 10-60 µg/ml, 50-300 µg/ml, the Molar absorptivity values are1.3874x 105, 2.9865x 104 L/mol.cm, Correlation Coefficients are 0.9988,0.9996, Sandell sensitivity are 3.9473x 10-3, 1.8337 x 10-2ng/cm2 .The methods proposed gave reproducible results with the percentage recoveries in the formulations found to be 99.812 to 99.749 and 99.682 to 99.774 for Methods A and B.

Keywords: Spectrophotometry; NQS; Bruci quinone, Per Iodate, Pharmaceutical formulations.

INTRODUCTION

Darunavir (DNV), (Figure 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 (3R,3aS,6aR)-hexahydro furo[2,3-b]furan-3-yl ester mono ethanoate [1] .

This drug is effective in patients experienced in anti-retroviral treatment, such as those carrying HIV-1 strains which are resistance to more than API [2]. 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 [3-4] 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 Spectroscopy method for determination of DNV in tablets [8]. Few methods have been developed for determination of DNV by HPLC [9-13]and Electrophoretic method for the separation of DNV [14]and Spectrophotometric methods [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 proposed methods. 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 throughout. Krishna Kumar Rao et al have estimated Darunavir Ethanolate by spectrophotometry [23]. The author investigated the role of various reagents used [26-29]. Upon thorough 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

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 (1 mg/ml) of DNV was prepared by dissolving 100 mg of it in 100 ml of milli pore distilled water. A portion of this stock solution was diluted stepwise with the distilled water to obtain the working standard DNV solution of concentrations 4-600 µg/ml.

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 (Figure 1). The quantity of the drug was computed from the Beer's law plot (Figure 2) of the standard drug in distilled water.

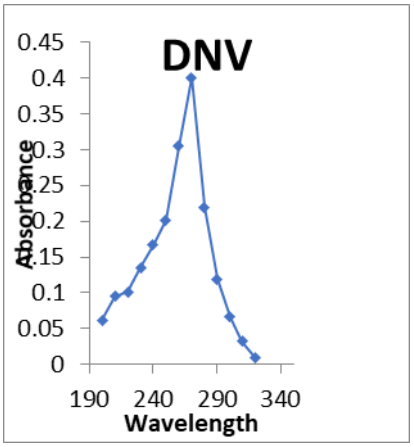


Figure 1: Absorption spectra of DNV in methanol (UV reference method)

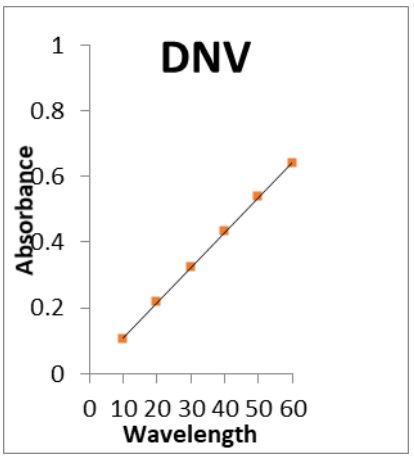


Figure 2: Beer's law Plot of DNV in methanol (UV reference method)

RECOMMENDED PROCEDURES

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.

Method-A

Aliquots of standard DNV solution (0.1-0.6 ml, 60 µg/ml) were placed into a series of 20 ml test tubes. Then 1.0 ml of NQS and 1.0 ml of NaOH solutions were added to each tube and kept aside for 2 min. at lab temperature. The solutions were made up to the mark with distilled water. The absorbances were measured at λmax 444 nm (Figure 3) against a reagent blank prepared simultaneously. The amount of DNV in a sample was obtained from the Beer-Lambert's plot (Figure 4).

Method-B

Into a series of 20 ml calibrated tubes, aliquots of standard DNV solution (0.1 – 0.6 ml, 300 µg/ml) were taken. Then 3.0 ml of brucine solution and 1.5 ml of IO4- solution were added successively. The flasks were kept aside for 2 min. and then 2.0 ml of Sulphuric acid was added and then heated the contents on boiling water bath for 15 min. The flasks were cooled to room temperature and made up to the mark with distilled water. The absorbance of the coloured species was measured after 5 min at λ max 519 nm (Figure 5) against the reagent blank. The amount of DNV was computed from its calibration graph (Figure 6).

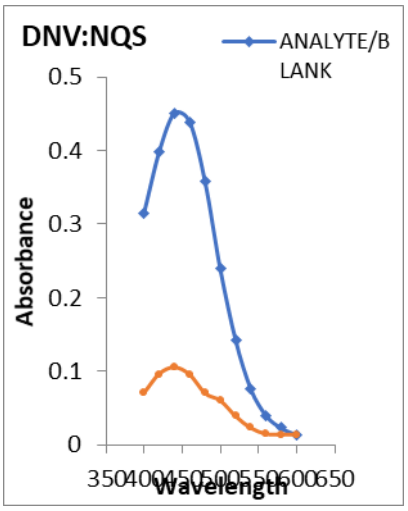


Figure 3: Absorption spectra of DNV: NQS

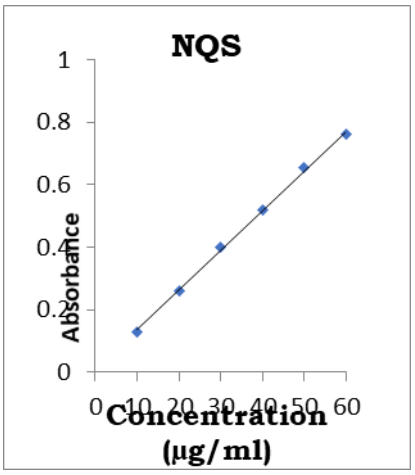


Figure 4: Beer's plot of DNV: NQS

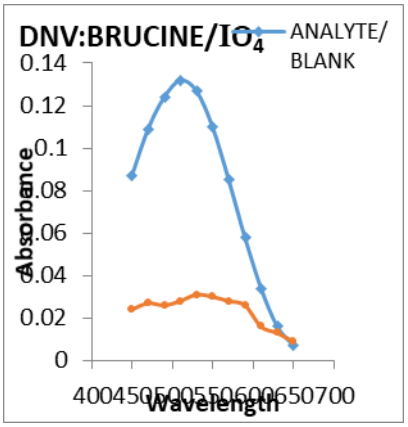


Figure 5: Absorption spectra of DNV: BRUCINE/IO_4-

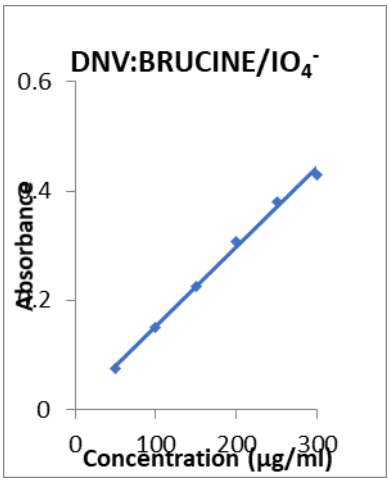


Figure 7: Beer's plot of DNV: BRUCINE/IO_{4-}

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

S. No	Parameter	Method-A	Method-B
1	Wave length λmax (nm)	444	511
2	Beer's law limits (µg ml^{-1})	Oct-60	50-300
3	Detection limits (µg ml^{-1})	2.057	9.5925
4	Molar absorptivity (1 mole cm^{-1})	1.3874x 10^5	2.9865x 10^4
5	Sandell's sensitivity(µg cm^{-2} / 0.001 absorbance unit)	3.9473x 10^{-3}	1.8337 x 10^{-2}
6	Regression equation (Y = a + bC)	0.0127	0.0027
	Slope (b)		
7	Standard deviation of slope (Sb)	2.2360 x 10^{-4}	4.4336 x 10^{-5}
8	Intercept (a)	0.0079	0.0065
9	Standard deviation of intercept (Sa)	8.7082 x 10^{-3}	8.6332 x 10^{-3}
10	Standard error of estimation (Se)	9.3541x 10^{-3}	9.2736 x 10^{-3}
11	Correlation coefficient (r^2)	0.9988	0.9996
12	Relative standard deviation (%)*	1.0079	1.3628
13	% Range of error(Confidence Limits) 0.05 level*	1.0579	1.4304
14	% Range of error(Confidence Limits)0.01 level	1.659	2.2433
15	% Error in bulk samples**	0.198	0.315

*: Average of six determinations considered**: Average of three determinations

Table 2: Assay and recovery of DNV in Pharmaceutical Formulations

Sample	Amount taken (mg)	Amount found by proposed methods		Reference Methods	Percentage recovery by proposed methods	
		Method-A	Method-B		Method-A	Method-B
Tablet	300	299.2	299.05	299.3	99.812	99.682
I		± 0.209	± 0.148	± 0.186	± 0.041	± 0.044
		F=1.26	F=1.57			
		t=1.50	t=1.40			
Tablet	300	299.17	299.3	299.25	99.749	99.774
II		± 0.343	± 0.518	± 0.401	± 0.110	± 0.068
		F=1.367	F=1.668			
		t=1.72	t=1.60			

* 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.

** After adding 3 different amounts of the pure labeled to the pharmaceutical formulations, each value is an average of 3 determinations.

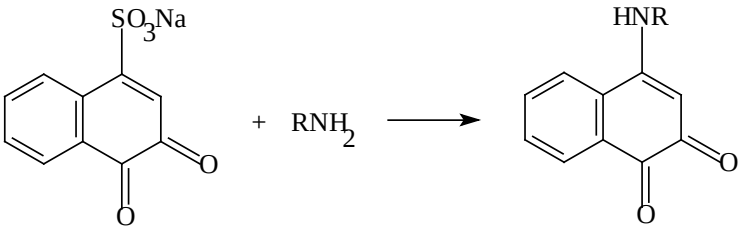
Chemistry of coloured species in the present investigation

DNV possesses different functional moieties such as primary amine, tertiary amine and sulphonyl groups of varied reactivity. Tertiary amine undergoes nucleophilic attack of dimethoxy benzene nucleus forms oxidative coupling with Brucine/IO_{4-}

Method-A

In this method, the presence of primary amino group in DNV permits the development of new spectrophotometric method for its determination through the formation of coloured nucleophilic substitution reaction product with NQS. The reactions of DNV with NQS are described in the scheme-1

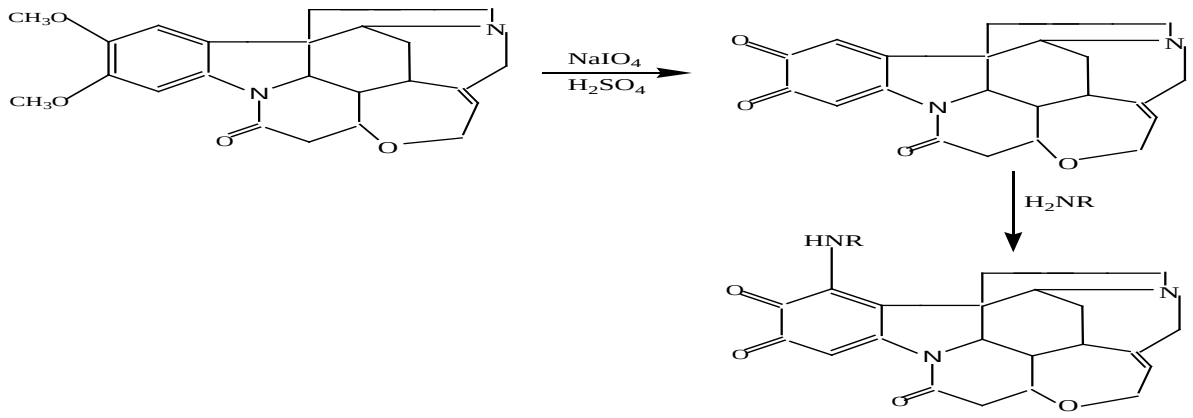
Scheme-1



Method-B

The dimethoxy benzene nucleus of brucine is attacked by IO_{4-} with the formation of O-quinone (Bruci quinone) which in turn undergoes nucleophilic attack on the most electron rich position of the coupler i.e., proton bearing amino group (primary amine), to give 1-mono substituted Bruci quinone derivative. The reactions of DNV with brucine in the presence of IO_{4-} are described in the scheme -2.

Scheme-2



RESULTS AND DISCUSSION

In developing the proposed methods, a systematic study of the effects of various relevant parameters in the methods concerned were undertaken by varying one parameter at a time and controlling all other parameters to get maximum color development, minimum blank color, reproducibility and the responsible period of stability of final colored species formed. In order to test whether the colored species formed (or diminished) in the above methods adhere to Beer-Lambert's plot, the absorbance at appropriate wavelength of a set of solutions containing different amounts of DNV and specified amounts of reagents (as described in the recommended procedures of each method) were noted against appropriate reagent blanks or distilled water. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. Beer-Lambert's limits, molar absorptivity and Sandell's sensitivity for DNV with each one of the mentioned reagents were calculated. The precision of each one of the two proposed visible spectrophotometric methods were ascertained separately from the absorbance values obtained by actual determination of eight replicates of a fixed amount of DNV in the final dilution. The present relative standard deviation and percent range of errors (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods are presented in the Table 1. The values obtained by the proposed and reference method (UV) for pharmaceutical formulations were compared statistically by the t-and F- test were given in Table 2 and found not to differ significantly. Recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical excipients present in pharmaceutical formulations. The proposed methods are found to be simple, sensitive and accurate and can be used for the routine quality control analysis of DNV in bulk and samples and pharmaceutical formulations.

CONCLUSION

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

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