



Condensation Methods for the Determination of Darunavir in Pure and Pharmaceutical Formulations

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Abstract : Two visible spectrophotometric methods were developed A and B for the determination of Darunavir in pure and pharmaceutical formulations. The methods are based on condensation reaction with PDAB (Method-A) and ONB (Method-B) in presence of acidic medium with the primary amine group in DNV. The coloured products exhibit absorption λ_{\max} at 639 nm and 452nm for methods A and B respectively. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 10-60 $\mu\text{g/ml}$, 50-300 $\mu\text{g/ml}$, correlation co-efficients are 0.9983, 0.9989; Sandell's sensitivities are 9.9833×10^{-3} , 3.0456×10^{-2} (1 mole cm^{-1}); and molar absorptivity values are 5.4857×10^4 , 1.7981×10^4 ($\mu\text{g cm}^{-2}$) for methods-A and B respectively. The proposed methods are applied to commercial available formulations and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the DNV in the presence of other ingredients that are usually present in formulations. These methods offer the advantages of rapidity, simplicity and sensitivity and low cost without the need for expensive instrumentation and reagents.

Key words : Condensation, PDAB, ONB, Regression Analysis.

Introduction and Experimental

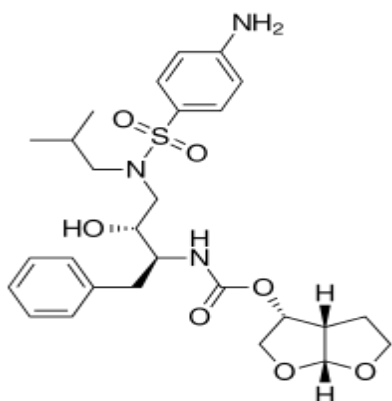


Fig.1 Structure of Darunavir

Darunavir (DNV) is an oral anti-retroviral agent (Fig.1) which selectively inhibits the cleavage of Human immunodeficiency virus (HIV-1) encoded Gags-polyproteins in infected cell, thereby preventing the formation of mature virus. Darunavirethanolate is chemically [(1*S*,2*R*)-3-[[[4-amino phenyl] sulfonyl](2-methyl propyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3*R*,3*aS*,6*aR*)-hexahydrofuro[2,3-*b*]furan-3-yl ester monoethanolate¹. Darunavir was designed to form robust interactions with the protease enzyme from many strains of HIV, including strains from the treatment-experienced patients with multiple resistance mutations to Protease inhibitors². It blocks HIV protease, an enzyme which is needed for HIV to multiply. According to *in vitro* experiments, DNV was active against HIV-1 with PI-resistance mutations and against PI-resistance clinical isolates^{3,4}. This drug is effective in patients experienced in anti-retroviral treatment, such as those carrying HIV-1 strains which are resistance to more than one PI⁵. The use of advanced instrumentation techniques for the analysis of drugs has been discussed elsewhere⁶. Literature survey revealed that different analytical methods have been reported for the determination of DNV in plasma using liquid chromatography coupled with tandem mass spectroscopy⁷ simultaneous determination of DNV with other anti-retroviral agents in plasma^{8,9}. Few HPTLC methods for determination of DNV in rat plasma and in tablet dosage form its application to pharmacokinetics studies¹⁰. Infrared Spectroscopy method for determination of Darunavir in tablets¹¹. Few methods had been developed for determination of DNV by HPLC¹²⁻¹⁷ and electrophoretic method¹⁸ for the separation of DNV. 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. Therefore the author has made attempt to develop rapid RP-HPLC method in part-B for determination and estimation DNV in bulk and tablet dosage form. Validation as per ICH and USFDA guidelines^{19,20} is done along with stress degradation study. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the development and validation of DNV in bulk drug samples and in pharmaceutical dosage form.

Instrument Used

A Shimadzu 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 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 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 further diluted to 25 ml to get 40 $\mu\text{g/ml}$ solution. The absorbance of the solution was determined λ_{max} 223 nm (Fig.5.01). The quantity of the drug was computed from the Beer's law plot (Fig. 5.02) of the standard drug in distilled water.

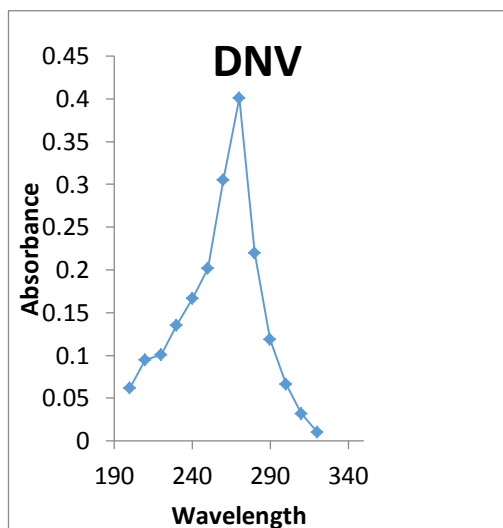


Fig.2 Absorption spectra of DNV in methanol (UV reference method)

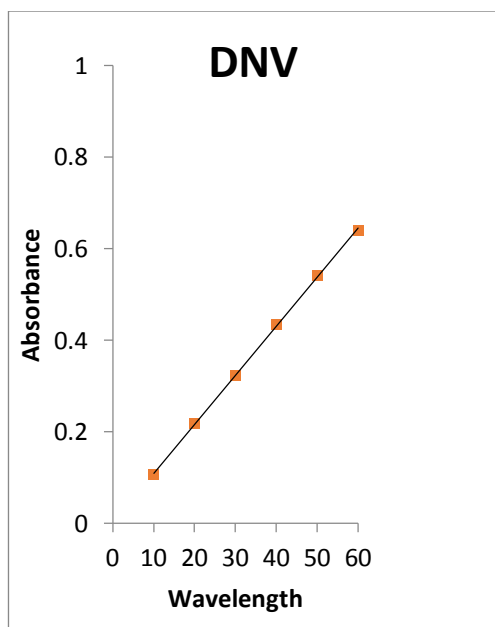


Fig.3 Beer's law Plot of DNV in methanol (UV reference method)

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

To each of 10 ml calibrated tubes, aliquots (0.1 – 0.6 ml, 60 $\mu\text{g/ml}$) of standard DNV solution, 2.0 ml of PDAB and 3.0 ml of conc. sulphuric acid were added successively and the total volume in each flask was brought to 9 ml by the addition of methanol and placed in a heating water bath for 15 minutes. Then the flasks were cooled and made up to the mark with methanol and absorbance was measured after 5 min. at λ_{max} 650 nm (Fig.4) against a reagent blank prepared in a similar way. The amount of DNV in sample solution was obtained from the Beers-Lambert's plot (Fig 5)

Method-B

Into a series of 10 ml calibrated tubes, aliquots of standard DNV solution (0.1-0.6ml, 60 $\mu\text{g/ml}$) 1ml of ortho nitro benzaldehyde, 2 ml of sulphuric acid and 2 ml of methanol was added successively and solution allowed to stand for 5 minutes. The solution made up to the volume by adding distilled water. The absorbance of each solution was measured at λ_{max} 451 nm (Fig.6) against reagent blank prepared in similar manner. The amount of DNV in the sample was computed from Beer's-Lamberts Law (Fig.7)

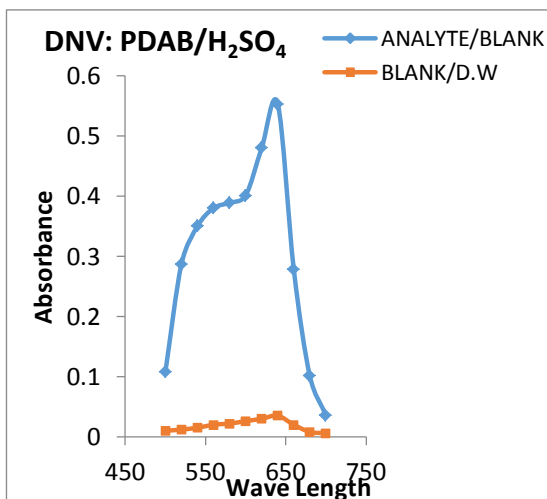


Fig .4 Absorption spectra of DNV:PDAB/H₂SO₄

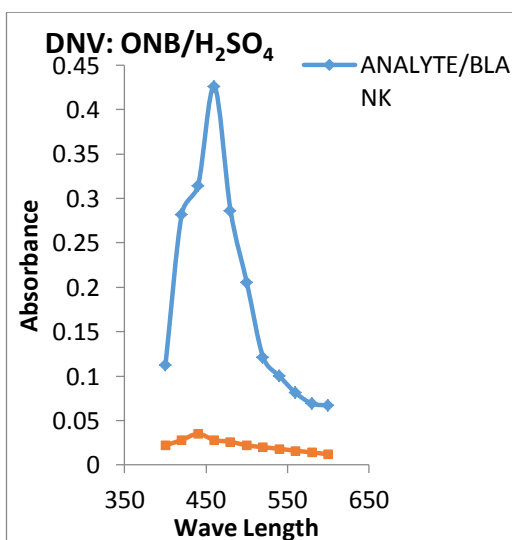


Fig 5 Absorption spectra of DNV: ONB/H₂SO₄

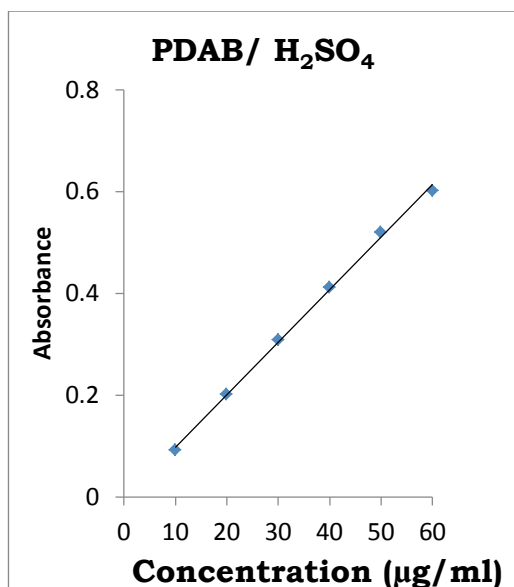
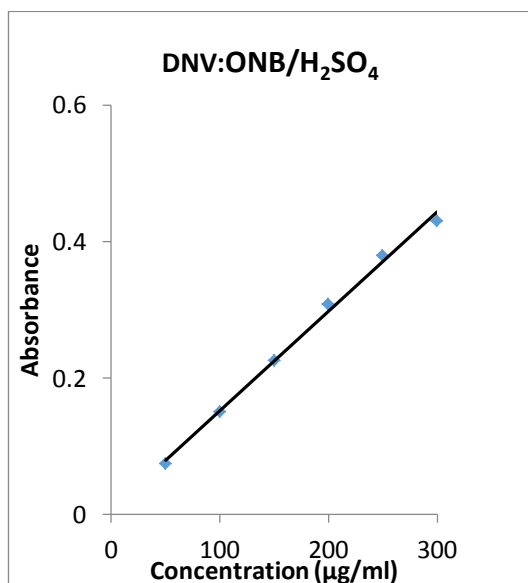
Fig.6 Beer's plot of DNV: PDAB/H₂SO₄Fig.7 Beer's plot of DNV: ONB/H₂SO₄

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

Sl.No	Parameter	Method-A	Method-B
1	Wave length λ_{\max} (nm)	639	452
2	Beer's law limits ($\mu\text{g ml}^{-1}$)	10-60	50-300
3	Detection limits ($\mu\text{g ml}^{-1}$)	2.4290	10.7554
4	Molar absorptivity (1 mole cm^{-1})	5.4857×10^4	1.7981×10^4
5	Sandell's sensitivity ($\mu\text{g cm}^{-2} / 0.001 \text{ absorbance unit}$)	9.9833×10^{-3}	3.0456×10^{-2}
6	Regression equation ($Y = a + bC$) Slope (b)	0.0103	0.0033
7	Standard deviation of slope (S_b)	2.1414×10^{-4}	6.0757×10^{-5}
8	Intercept (a)	-0.0049	0.0029
9	Standard deviation of intercept (S_a)	8.3396×10^{-3}	1.1831×10^{-2}

10	Standard error of estimation (S_e)	8.9582×10^{-3}	1.2708×10^{-2}
11	Correlation coefficient (r^2)	0.9983	0.9989
12	Relative standard deviation (%) [*]	1.0083	0.2496
13	% Range of error(Confidence Limits) 0.05 level [*]	1.0583	0.2620
14	% Range of error(Confidence Limits)0.01 level	1.6598	0.4109
15	% Error in bulk samples ^{**}	0.137	0.1

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

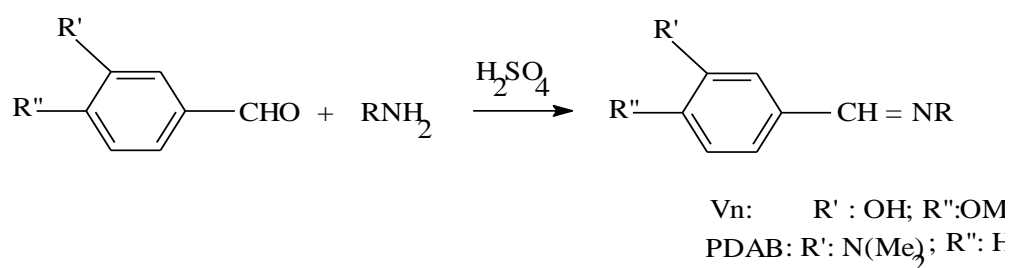
Table:2. Assay and recovery of DNV in Pharmaceutical Formulations

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 I	300	299.12 ±0.175 F=1.27 t=0.42	299.25 ±0.225 F=1.466 t=1.05	299.3 ±0.186	99.802 ±0.054	99.767 ±0.079
Tablet II	300	299.15 ±0.378 F=1.12 t=1.84	299.20 ±0.408 F=1.034 t=0.96	299.25 ±0.401	99.270 ±0.084	99.573 ±0.103

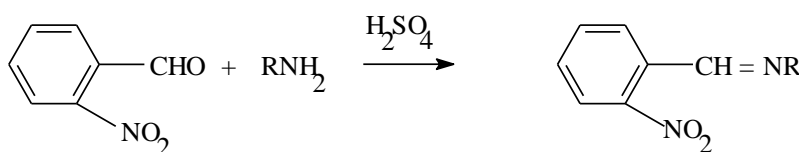
Chemistry of coloured species in the present investigation

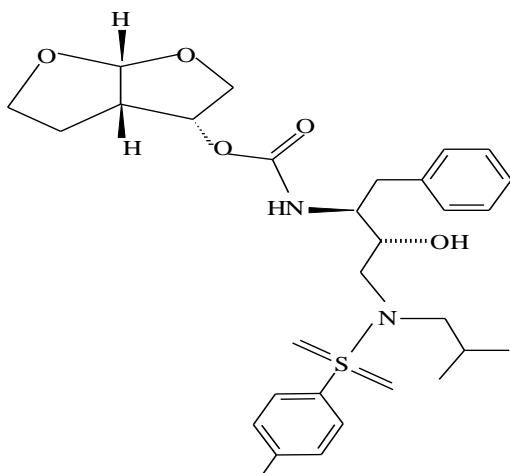
DNV possesses different functional moieties such as primary amine, tertiary amine and sulphonyl groups of varied reactivity. The methods are proposed here based on condensation reaction with PDAB (Method-A) and ONB (Method-B) in presence of acidic medium. In the present investigation, it is observed that under acidic conditions the primary group in DNV forming coloured condensation product with aromatic aldehyde PDAB(Method-A), ONB (Method-B)] in the presence of H_2SO_4 in non aqueous medium. The nature of coloured species obtained with PDAB and ONB are represented in the schemes 1,2 respectively.

Scheme.1



Scheme.2





For above all

Results and Discussion

Optimum operating conditions used in the procedure were established adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as were studied. Volume of PDAB, volume of conc. H_2SO_4 on colour development, effect of order of addition of reagents on colour development, effect of temperature ($^{\circ}C$) shaking time (min), solvent for final dilution, stability period after final dilution were studied for method-A, and effect of volume of ONB required for condensation, volume of H_2SO_4 , effect of CH_3OH , reaction time and stability period after final dilution were studied for method-B.

The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements, Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in **Table-1**. Commercial formulations containing DNB were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-test and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pure analyzed formulations at three different concentration levels. These results are summarized in **Table-2**.

Conclusion

The proposed methods for DNV determination have many advantages over other analytical methods due to its rapidity, lower cost and environmental safety. Unlike HPLC, LC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are in expensive and available in any analytical laboratory. The proposed methods report new ways for the determination of DNV in pharmaceuticals

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