IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2018 Vol.:12, Issue:2

Analytical Method Development for the Estimation of Darunavir by Ion-Pair Complex Using Visible Spectrophotometry

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

R. Vijayalakshmi*[1], D. Anjani[1], M. D. Dhanaraju[1]

*[1]*Research Lab, GIET School of Pharmacy, NH-16 Chaitanya Knowledge City Rajahmahendravaram-533296. Andhra Pradesh*

Submission: 22 April 2018
Accepted: 28 April 2018
Published: 31 May 2018

Keywords: Darunavir; Bromo cresol green; Bromothymol blue; Validation.

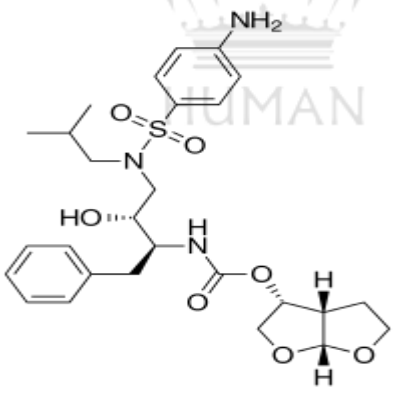
ABSTRACT

Objective: This proposed work describes two simple, fast and extractive colorimetric methods for the estimation of Darunavir ethanolate with BCG (method A)/BTB (method B) in both bulk and tablet formulations. **Methods:** Both the methods were developed on Perkin Elmer (LAMBDA 25) UV–Visible Spectrophotometer interfaced with UV Winlab software and 1cm quartz cells. These methods focus on the formation of colored ion pair complex of Darunavir with acidic dyes (BCG/BTB). The methods were optimized as per standard optimization parameters. **Results:** The yellow colored products of Darunavir were quantified at 418 nm and 411nm with BCG and BTB, respectively. The linear relationship was observed between absorbance and the corresponding concentration of drug in the range of 20-140µg/ml and 40-140µg/ml, respectively for method A and B. The colorimetric methods were extensively validated as per ICH guidelines and all the parameters were within the acceptance criteria, with the correlation coefficient of 0.9999 and % RSD less than 2 for both the methods. The results of the accuracy studies were nearer to 100%.**Conclusion:** The methods were proved to be more accurate, simple, precise and rapid by statistical validation as well as recovery studies and could be used for routine analysis.

INTRODUCTION

Darunavir(DNV)[1,2], chemically is [1S,2R]-3-[[(4aminophenyl)sulfonyl] (2-methyl propyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid(3R,3aS,6aR)hexahydrofuro[2,3-b]furan-3-yl ester mono ethanolate (fig.1). Its molecular formula is $C_{27}H_{37}N_3O_7S.C_2H_5OH$ and the molecular weight is 593.73 g/mol. It is a protease inhibitor that prevents HIV replication by binding to the enzyme's active site, thereby preventing the dimerization and the catalytic activity of the HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in virus-infected cells, which prevents the formation of mature infectious virus particles.

Literature survey of DNV revealed that there are very few RP-HPLC[1-6], spectrophotometric[7-10], and HPTLC[11] methods available for the quantization of DNV in pure and dosage forms. Both HPLC and HPTLC methods are high in cost, time-consuming and need sample pretreatment. The present work describes two simple, time-effective, cost-effective and sensitive methods based on the ion association complex process for quantization of DNV in pure and tablet dosage forms.



.C_2H_5OH

Fig. 1: Structure of DNV

MATERIALS AND METHODS

Equipment

Double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer interfaced with UV WIN lab software and 1 cm quartz cuvettes were used for spectral measurements. Sartorius balance was used for weighing the samples.

Chemicals

DNV was obtained as a gift sample from Aurobindo Pharma Ltd, Hyderabad. Ethanol, Bromo cresol green, Bromothymol blue, Hydrochloric acid, and Chloroform were used for the experimental work. All the chemicals used in experimental work are AR grade.

Experimental

Preparation of stock solution of DNV

25 mg of DNV was accurately weighed and transferred to a 25 ml volumetric flask, dissolved and diluted to final volume with ethanol. The resulting solution has a concentration of 1 mg/ml.

Preparation of reagents

(0.1% w/v) Bromo cresol green (BCG)

100 mg of BCG was weighed and dissolved in 1ml of 0.1N NaOH and 5ml of methanol and final volume was made up with water.

(0.1% w/v) Bromothymol blue (BTB)

100 mg of BTB was weighed and dissolved in 10 ml of 4% NaOH and 20ml of alcohol and dilute to 100ml with distilled water.

(0.2N)HCl

0.85 ml of Conc. HCl was measured and transferred to a 50 ml volumetric flask then made up with double distilled water.

Procedure for calibration standards (Method A)

In a series of 10 ml volumetric flasks, 0.2-1.4 ml of working standard solution of DNV was pipetted out and transferred to a separating funnel. Then 0.5ml of hydrochloric acid and 0.6ml of BCG and 2.5 ml of chloroform were added. The contents were shaken vigorously and kept aside for 5 min. The yellow colored chloroform layer was separated and the extraction was repeated twice with 2.5 ml of chloroform. The combined chloroform extracts were measured at 418 nm against the reagent blank.

Procedure for calibration standards (Method B)

In a series of 10 ml volumetric flasks, 0.4-1.4 ml of working standard solution of DNV was pipetted out and transferred to a separating funnel. Then 2ml of hydrochloric acid, 2ml of BTB and 2.5ml of chloroform were added. The contents were shaken vigorously and kept aside for 5 min. The yellow colored chloroform layer was separated and the extraction was repeated twice with 2.5 ml of chloroform. The combined chloroform extracts were measured at 411nm against the reagent blank.

The assay procedure for Method A/Method B

20 tablets of commercial samples of DNV were accurately weighed and powdered. Tablet powder equivalent to 25 mg was weighed. Then dissolved and diluted to volume with 25 ml of ethanol and filtered. Then the solution was subjected to the procedure as described above and the absorbance was measured at 418nm, 411nm for the method A and method B, respectively.

RESULTS AND DISCUSSION

Optimization of the Method

The spectral characteristics of both the methods using BCG and BTB reagents were performed by optimizing the methods for several optimization parameters. Fig 2 and 3 represents absorption spectra of DNV by Method A and Method B, respectively.

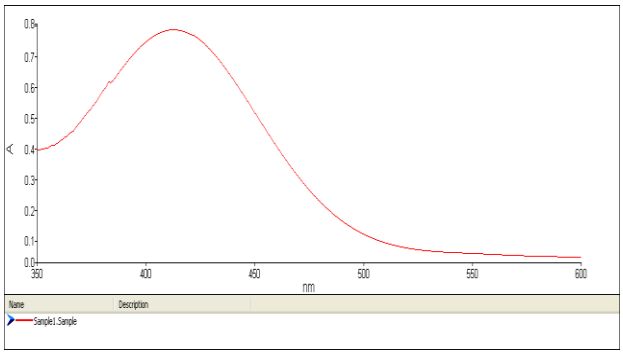


Fig 2: Absorption spectrum of DNV by method-A

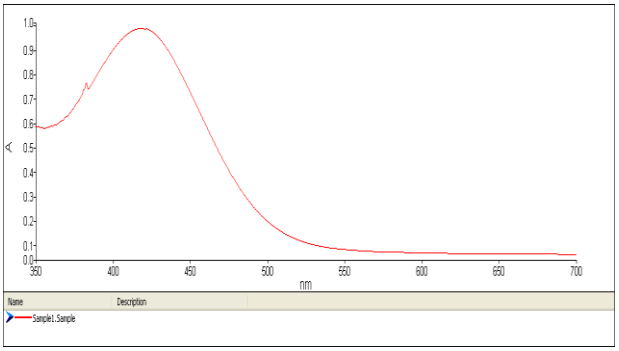


Fig 3: Absorption spectrum of DNV by method-B

Order of Addition and reagent concentration

To find out whether the order of addition has any influence, the absorbance of a set of solutions prepared by mixing the reagents and the amounts of drug levels in different sequences, as given in the recommended procedure was measured, studied and presented in table 1. Effect of concentration of reagents was studied and shown in table 1.

Table 1: Fixing order of addition for method A and method B

Method	Order of Addition	Absorbance
A	DNV + HCl(0.5 ml) + BCG(0.6ml)	1.541
B	DNV + HCl(2ml) + BTB(2ml)	0.898

Effect of temperature, the effect of time and stability of colored products

Effect of temperature on reaction conditions was studied; if the temperature is maintained above 40^{o}C the intensity of absorbance reduces; lowering the temperature has no effect on absorbance. Hence these methods were carried out at room temperature. The formation of the colored complex was complete in 5 min time interval at room temperature. The stability of colored product was studied by taking the absorbance at various time intervals and the color of the reaction products was stable for 2 h. The scheme of reaction for the selected drug was given in Fig 4 and 5.

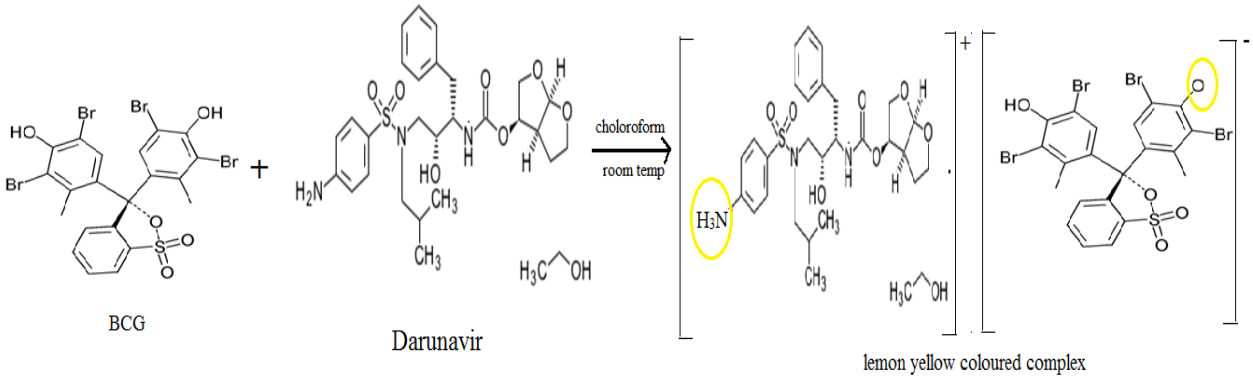


Fig 4: Reaction scheme with BCG

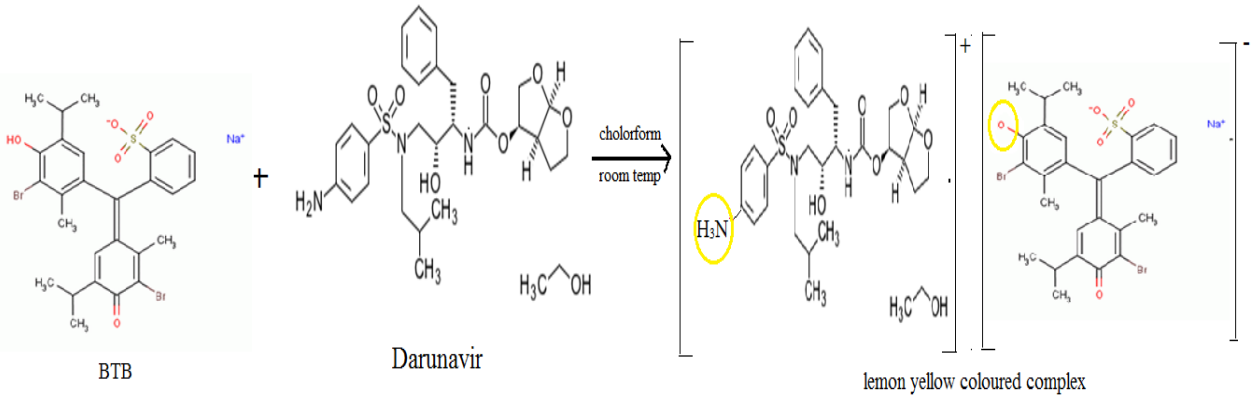


Fig 5: Reaction scheme with BTB

Method validation

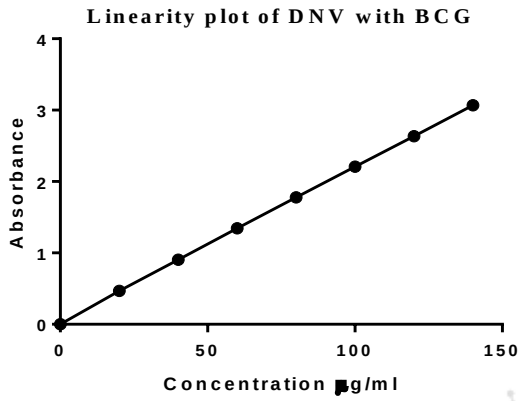
All the methods were validated as per ICH guidelines[12] for accuracy, precision, linearity, LOD, LOQ, ruggedness and robustness and the results were found to be satisfactory. The regression parameters were presented in table 2.

Table 2: Optical and regression parameters for method A and method B

PARAMETERS	Method A	Method B
$\lambda_{max,}$ nm	418	411
Beer's law range(µg/ml)	20-140	40-140
Molar extinction coefficient (L.mole^{-1} .cm^{-1})	1.32x10^{5}	5.87x10^{4}
Sandell' s sensitivity (µg/cm^{2})/0.001 abs unit	8.09x10^{-2}	7.2x10^{-2}
LOD, µg/ml	1.8767	1.4141
LOQ, µg/ml	6.2557	4.7138
Slope (m)	0.02182	0.009820
Intercept (b)	0.02417	0.005578
The correlation coefficient (r)	0.9999	0.9999

Linearity and range

At the described experimental conditions for DNV standard calibration curves were constructed by plotting an increase in absorbance with concentration. Table 4, Fig 6&7 shows linear correlation found between absorbance and concentration of DNV. The statistical parameters given in the regression equation were calculated from the calibration graphs. The high values of the regression coefficients and low values of y-intercepts of the regression equations proved the linearity of the calibration curves.



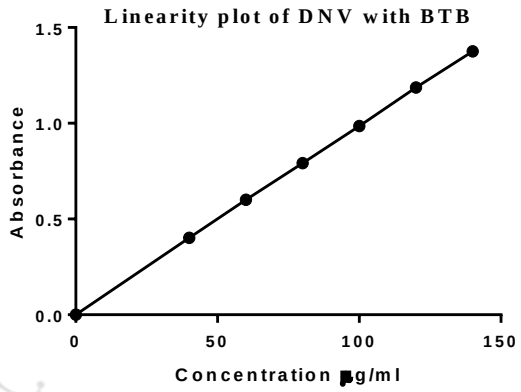


Fig 6: Linearity plot of DNV with BCG Fig 7: Linearity plot of DNV with BTB

Precision

The precision of the proposed methods was assessed by determining the relative standard deviation (RSD) of six replicate analyses on the same solution containing a fixed concentration of DNV (within Beer's law limit). The low % RSD of the intraday and interday repeatability studies corroborate precision of the method. Table 3 represents the results of precision studies.

Table 3: Results of precision studies

Parameter	Method A		Method B	
	Intraday*	Interday*	Intraday	Interday*
Conc,(µg/ml)	80		100	
Mean abs	1.7853	1.7873	0.9866	0.9923
SD	0.00049	0.00049	0.00049	0.00049
% RSD*	0.02755	0.02757	0.0498	0.0495

**N= average of six determinations*

Robustness and Ruggedness

Robustness was ascertained by the low % RSD through narrow alteration of the optimized parameters. System to system/analyst to analyst variability study was conducted for ruggedness studies. The % RSD was found to be less than 1 by both studies which corroborate the method is rugged and robust.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined by analyzing progressively lower concentrations of standard solution using optimized conditions and the results were presented in table 2.

Accuracy

The validity and accuracy of the proposed methods were further assessed by recovery studies using the standard addition technique. For this purpose, a known amount of pure drug at three different levels was spiked to the fixed and known quantity of pre-analysed formulation samples and the concentration of the drug was estimated by the proposed methods. The results are given in Table 4 establish that the methods were reproducible by low SD and % RSD. No interference was evidenced from the common formulation excipients.

Table 4: Accuracy table for method A and method B

Method	Std amount added	Drug in formulation	%Recovery	SD	%RSD N=3
A	60	40	99.683	0.00577	0.00579
		60	99.773	0.00577	0.00578
		80	99.826	0.00577	0.00578
B	60	40	99.486	0.00577	0.0058
		60	99.746	0.00577	0.00578
		80	99.923	0.00577	0.00577

Application of the proposed method to the formulation

The proposed methods were applied to the determination of DNV in tablet formulations. The recoveries are close to 100% indicating that there is no serious interference in samples. The good agreement between these results and known values indicate the successful applicability of the proposed methods for the determination of DNV in formulations. The results are given in Table 5.

Table 5: Assay results for method A and method B

Methods	Formulation	Label claim (mg)	Amount found (mg)	%Recovery
Method A	Darunavir (Cipla Ltd)	300	299.09	99.69
Method B		300	299.40	99.80

CONCLUSION

The new, cost-effective, simple and sensitive visible spectrophotometric methods, using BCG and BTB were developed for the determination of DNV in bulk and in pharmaceutical formulations. The developed methods were also validated. From the statistical data, it was found that the proposed methods were accurate, precise and reproducible and can be successfully applied to the analysis of the same and could make a better alternative to the existing methods.

REFERENCES

1 Raveendra Babu G, Lakshmana Rao A, Venkateswara Rao J. Development and validation of novel HPLC method for estimation of darunavir in pharmaceutical formulations. Int J Res Pharm Chem 2013, 3:438-443.

2 Reddy BV, Jyothi G, Reddy BS, Raman NV, Reddy KS, Rambabu C. A novel stability-indicating reversed-phase high-performance liquid chromatographic (HPLC) method has been developed for the quantitative determination of Darunavir ethanolate, an HIV-1 protease inhibitor. J Chromatogr Sci, 2013; 51:471-76.

3 Satyanarayana L, Naidu SV, Narasimha Rao M, Alok Kumar, Suresh K. A reverse phase HPLC method was developed for the estimation of Darunavir in tablet dosage form. Asian J Res Pharm Sci, 2011; 1:74-76.

4 Manisha M, Pranali JG, Anuja VP, Mashish S. RP-HPLC Method for determination of darunavir in bulk and pharmaceutical preparations. Int J Pharm Sci Rev Res, 2013; 21:20-23.

5 Patel BN, Suhagia, Bhanubhai N, Chaganbhai NP. RP-HPLC method development and validation for estimation of Darunavir ethanolate in tablet dosage form. Int J Pharm Pharm Sci, 2012; 4:270.

6 Mastannama SK, SaiSirisha V, Alekhya G, Haritha K, Arunbabu V. New validated RP-HPLC method for estimation of darunavir in bulk and dosage form. Int Res J Pharm, 2014; 5:13-6.

7 Krishna Kumar Rao KVV, Phanindra B, Rajesh K. Spectrophotometric method for estimation of Darunavir ethanolate by using MBTH reagent in bulk and pharmaceutical dosage form. Inventi Rapid: Pharm Analysis & Quality assurance, 2013; 4:1-3.

8 Mastanamma SK, Sai Sirisha V. Validated visible spectrophotometric method for estimation of Darunavir in bulk and pharmaceutical dosage form using 1, 2 naphthoquinone 4-sulphonate reagent. World Journal of Pharmaceutical Research, 2014; 3:4615-24.

9 Vijayalakshmi R, Anjani D, Suvarna Pushpa Kumari S, Bhuvaneswari S, Dhanaraju MD Oxidative coupling method for the estimation of Darunavir and Capecitabine by visible spectrophotometry. Int J Pharm Tech Res, 2016; 9:146-154.

10 Vijayalakshmi R, Naga Sri Ramya Y, Dimple Mani A, Dhanaraju MD. Spectrophotometric determination of Darunavir Ethanolate by condensation technique. Int J Pharm Tech Res, 2016; 9:301-06.

11 Deshpande BP, Santosh RB. Development and validation of stability-indicating HPTLC method for determination of Darunavir ethanolate and Ritonavir. Int J Pharm Pharm Sci, 2015; 7:66-71.

12 ICH-Q2B Validation of analytical procedures: Methodology International Conference on Harmonization of Technical Requirements for registration of pharmaceuticals for human use, Geneva, Switzerland, 1996.