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UV-Spectrophotometric Determination of Daurnavir in Bulk and Pharmaceutical Dosage Form Using Hydrotropic Solubilization Technique (8m Urea)

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ABSTRACT

Hydrotropicsolvents may proper choice to preclude the use of organic solvents so that, a simple, accurate, novel, safe and precise method could developed for estimation of poorly water soluble drug,. Solubility of Daurnavirincreased by using 8M urea as a hydrotropic agent. Daurnavir1showed the maximum absorbance at 263.91nm in method A,254-274 nm in method B and 264nm in method C. At these wavelengths, hydrotropic agent and other tablet excipients did not show any significant interference in the spectrophotometric assay. The developed methods were found to be linear in the range of5-40 µg/ml for method A, method B&C with correlation coefficients (R) of 0.997, 0.995 and 0.997 respectively.The mean percent label claim of tablets of of Daurnavir in formulation estimated by the proposed methods was found to be107%. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical parameters were found to be good accordance with the prescribe values. As hydrotropic agent was used in the proposed methods, these methods were eco-friendly and it can be used in routine quantitative analysis of drug in bulk and dosage form in industries.

Key words: Daurnavir, urea; AUC; Hydrotropicsolubilization technique; derivative spectroscopy.

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INTRODUCTION

The term hydrotropic agent was first introduced by Neuberg (1916), to designate anionic organic salts which, at high concentrations, considerably increase the aqueous solubility of poorly soluble solutes. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. Hydrotropic agents consist generally of two essential parts, an anionic group and hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is prerequisite for a hydrotropic substance. On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization. Hydrotropes commonly used includes sodium benzoate, sodium acetate, sodium salicylate, nicotinamide, urea, trisodium citrate, sodium ascorbate, piperazine, caffeine, potassium citrate etc. hydrotropic agents have been observed to enhance the solubility of various substances in water. Darunavir is chemically (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)(4-aminobenzene) sulfonamido]-1-phenylbutan-2-yl]carbamate. It is white to off-white powder that is very slightly soluble in water and soluble in methanol[1, 2]. DRN is generally co-administered along with Ritonavir (100mg). Darunavir is an inhibitor of Dimerisation and the catalytic activity of the HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in the virus infected cells, thereby preventing the formation of infectious virus particles. Literature survey revealed that very few methods have been reported for the analysis of Daurnavir. Which include UV spectroscopy, [3, 5, 6]. Visible spectroscopy[4]. Reverse Phase High performance Liquid Chromatography[1], HPTLC, Titrimetric methods,NMR[7] and USP, BP. The present study illustrate development and validation of simple, economical, selective, accurate, precise spectrophotometric method for the determination of Daurnavir by using hydrotropic solubilization technique in bulk and Pharmaceutical dosage forms and validated as per ICH guidelines.

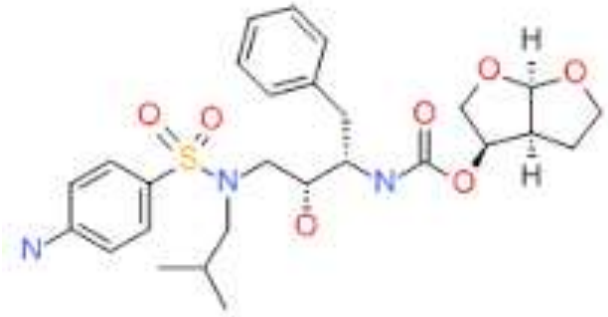

Figure 1 structure of Daurnavir

MATERIALS AND METHODS

Chemicals and reagents

Daurnavir (99.4%) was obtained as gift sample from protect laboratories, Hyderabad, India. Urea (A.R Grade; Qualigens) and distilled water used for the study.

Instrumentation

Shimadzu UV -1800 double beam spectrophotometer with 1cm path length supported by shimadzu UV-probe software ,version 2.21 was used for spectral measurements with 10mm matched quartz cells . Shimadzu balance (BL-220H) was used for weighing.

Selection of solvent

8M urea solution was used as a solvent for developing spectral characterstics of a drug. The selection was made after assessing the solubility in different hydrotropic solvents like sodium acetate, sodoim benzoate, piperazine, sodium chloride, citric acid. Among these solvents Daurnavir was freely soluble (1 in 10 parts as per IP-2010) in 8 M urea and showed maximum drug stability.

Preparation of reagent solution

8M urea solution was prepared by 48.6gm of urea pure chemical was weighed and dissolved in 10 ml distilled water and the volume was made upto the mark with distilled water in 100 ml volumetric flask.

Preparation of standard stock solution

Working standard Daurnavir 10 mg was weighed accurately and transferred to a 10 ml volumetric flask and add 5ml of 8M urea solution and heated at 70ºc for 15 mins on water bath. Volume was made up to the mark withdistilled water to give a solution of 1000µg/ml. It was further diluted with distilled water to get the concentration of 100µg/ml. from this solution a series of aliquots were prepared for further method development.

Absorption maxima method

For the selection of analytical wavelength 10µg/ml solution of Daurnavir was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectrum$\lambda_{max}$of Daurnavir, 263.91m was selected for the analysis. The calibration curve was prepared in concentration range of 5-40µg/ml at 263.91 nm. The calibration curve for Daurnavirwas plotted in the concentration v/s absorbance and regression equation was calculated. (Figures 2 & 3)

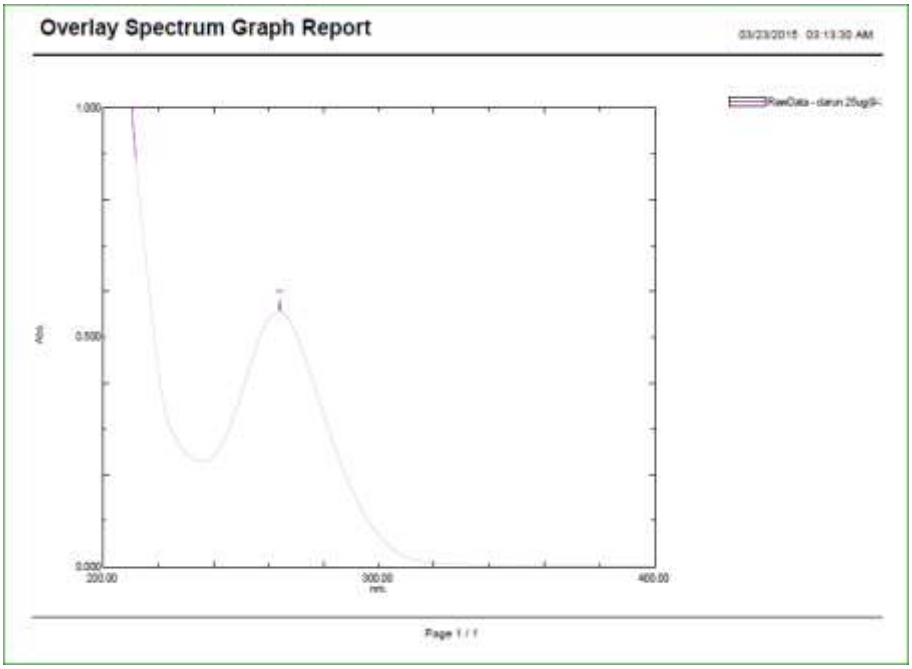

Figure 2: Absorption maxima spectrum ofDaurnavir

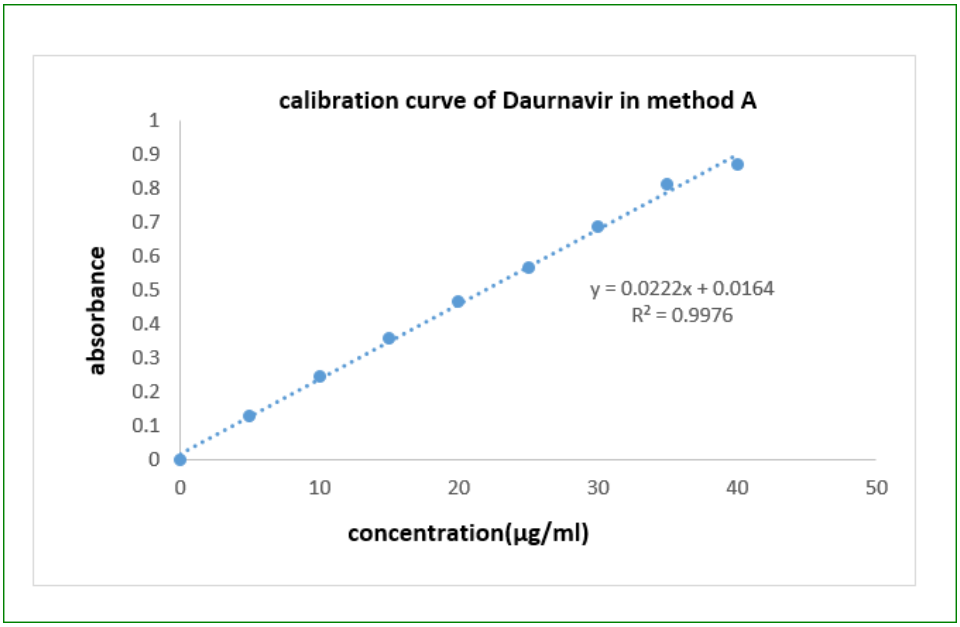

Figure 3: calibration curve of Daurnavir in method A

Area under curve method

For the selection of analytical wavelength 10µg/ml solution of Daurnavir was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. Area under curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 254-274 nm. Area calculation processing item calculates area bound by curve and horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The

wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. From this regression equation was calculated for the determination of amount of Daurnavir tablet formulation. (figures 4 & 5)

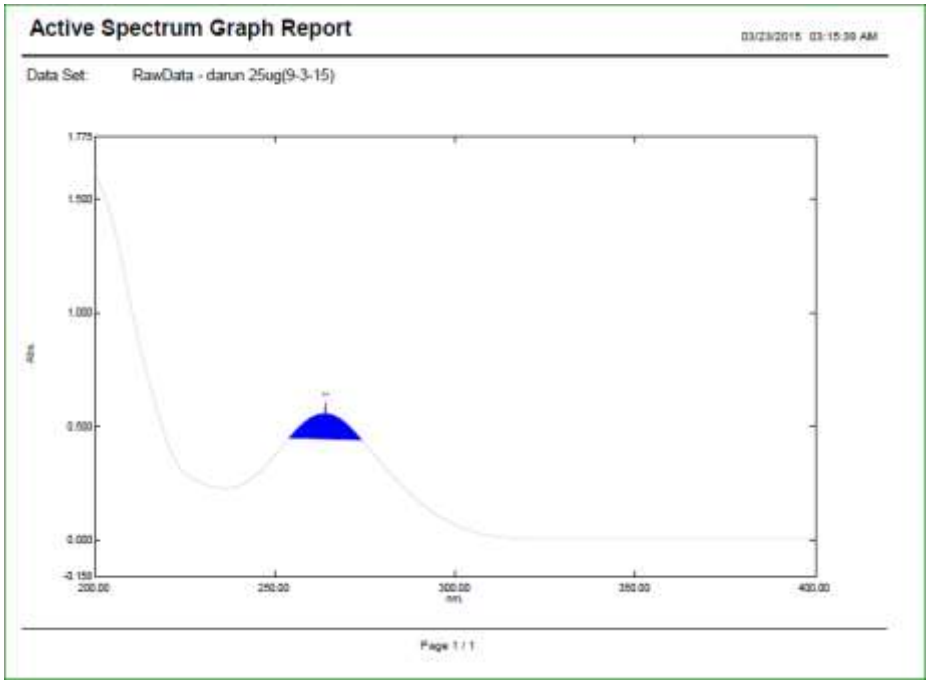

Figure 4: AUC spectrum of Daurnavir

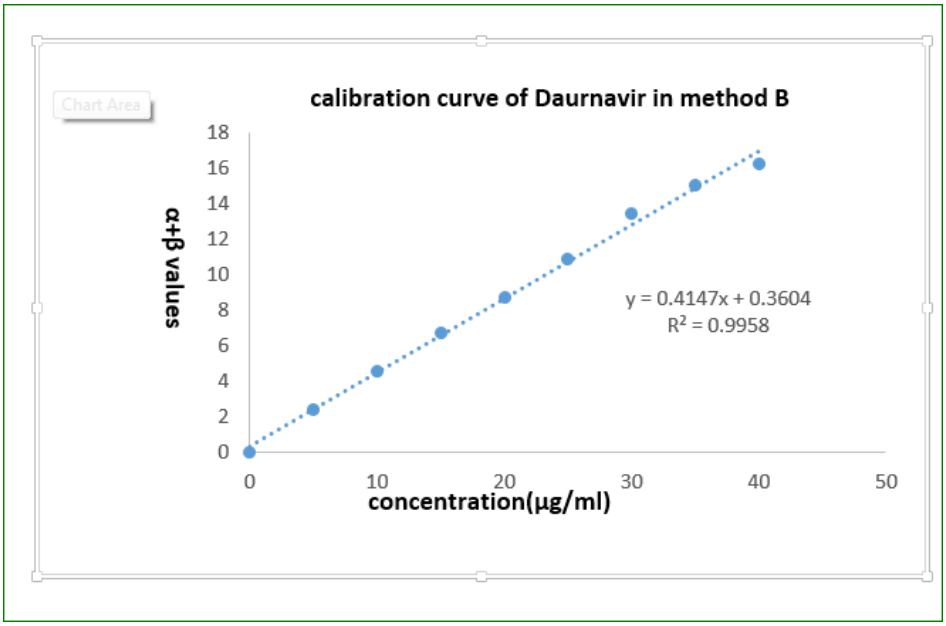

Figure 5: Calibration curve of Daurnavir in method B

First order derivative spectroscopy

It involves the conversation of normal spectrum to its zero, first, second or higher derivative spectrum. In derivative spectrophotometry, spectra are obtained by plotting the first or a higher order derivative of absorbance with respect to wavelength as a function of wavelength. Often,

these plots reveal spectral detail that is the lost in an ordinary spectrum. In addition, concentration measurements of an analyte in the presence of interference or of two or more analytes in a mixture can sometimes be made more easily accurately using derivative methods. In this method, 10µg/ml solution of Daurnavir was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200-400 nm. The absorption spectra thus obtained were derivitised from zero to second order. First order derivative spectra of drug showed a sharp peak at 264 nm, which was selected for its quantification. (Figures 6&7)

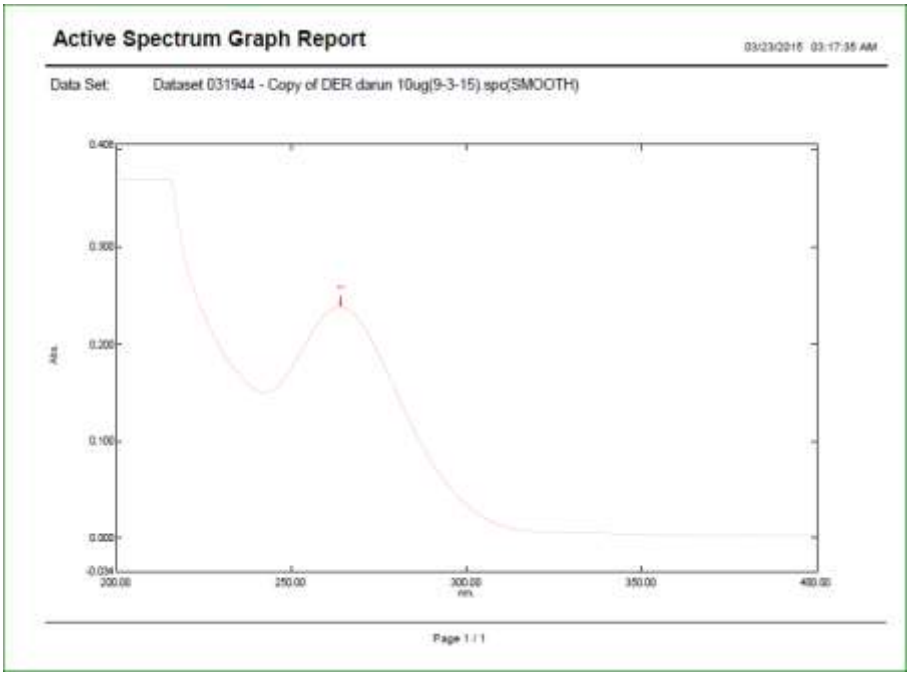

Figure 6: First order derivative spectrum of Daurnavir

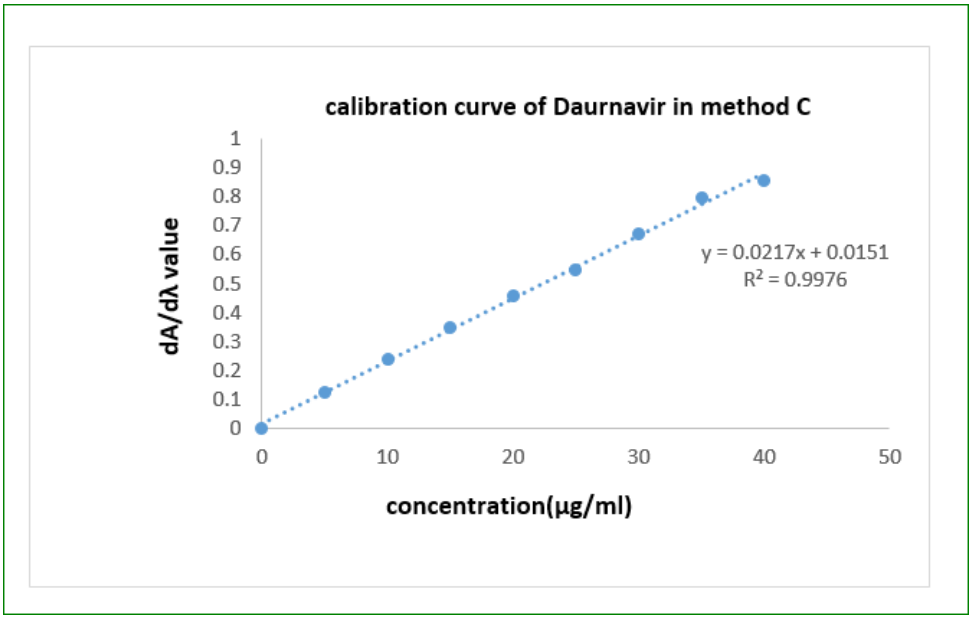

Figure 7: Calibration curve of Daurnavir in method C

Estimation of Daurnavir in laboratory mixture

For the estimation of Daurnavir laboratory synthetic mixture was prepared with daurnavir API and excipients with the strength of 300 mg. from this a quantity of powder equivalent to 10 mg of Daurnavir was transferred to10 ml volumetric flask and add 5 ml of 8M Ureasolution and heted at 70ºc for 15 mins on water bath, and make up the final volume with distilled water.It was filtered with Whatmann filter paper no.41 to obtain a stock solution of 1000 µg /ml of daurnavir, further dilutions of the stock solution were made in distilled water to get required concentration. In method A the concentration of Daurnavir was determined by measuring absorbance of sample solution at 363.91 nm .in method B, the concentration of Daurnavir was determined by measuring absorbance of sample solution in wavelength range of 254-274 nm. Inmethod C, first order derivative spectroscopy the concentration of Daurnavir was determined by measuring amplitude difference at λ_{max}264nm.

Method validation

The method was validated according to ICH guidelines to study accuracy,linearity and precision.

Linearity

In order to find out linearity range of proposed UV-spectrophotometric method, studies were carried out by plotting absorbance of analyte against concentrations of the analyte.A good linear relationship (r^2=0.997, 0.995 &0.997 for method A,B&C respectively) was observed between concentrations of Daurnavir and the corresponding absorbance.

Accuracy

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (2.5µg, 5µg, 7.5µg) of bulk sample to the pre-analyzed formulation .the solutions were suitably diluted in the range and then each of the dilution was observed 6 times.

Precision

Precision is the level of repeatability of results as reported between samples analyzed on the same day (intra-day) and samples run on 3 different days (inter-day).to check the intra-day and inter-day variation of the method, solution containing 5 µg/ml Daurnavir were subjected to the proposed spectrophotometric method of analysis and the recoveries obtained were noted. the precision of proposed method i.e. the intra and inter-day variations in the absorbance of the drug solutions.

LOD

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. The detection limit is usually expressed as the concentration of analyte.

The standard deviation and response of the slope-

$$LOD=3.3 * \text{standard deviation } (\sigma)/ s$$

LOQ

The quantitation limit of an analytical procedure is the lowest amount of an analyte of a simple which can be quantitatively determined with suitable precision and accuracy.

The standard deviation and response of the slope-

$$LOQ=10* \text{standard deviation } (\sigma)/ s$$

RESULTS AND DISCUSSIONS

For quantitative estimation of Daurnavir in bulk and tablet dosage form three validated methods was proposed for method A, the absorbance maxima was found to be263.91nm, for method C λ_{max} at 264nm was selected and for method B area under curve in the range of 254-274nm were selected for the analysis. Result of tablet analysis are shown in table 1 the assay procedure was repeated 6 times (n=6) (table.1)

Table 1: Analysis of daurnavir in laboratory synthetic mixture

Proposed methods	Label claim	Test conc(µg/ml)	Amount found (µg/ml)	%Assay	%RSD
A	300 mg	20	22.96	114.81%	0.325
B	300 mg	20	20.90	104.51%	0.656
C	300 mg	20	20.38	101.90%	0.745

The % assay by the three methods was found to be 114.81% in method A, 104.51% in method B and 101.90 % in method C. No interference was observed from the pharmaceutical excipients. The regression of Daurnavir concentration over its absorbance was found to be y=0.022X+0.016, Y=0.414x-0.360, Y=0.021x+0.015 for method A, B&C (where y is the absorbance and x is the concentration of Daurnavir).the slope,intercept and the correlation coefficient of the drug were shown in table.2 The % recovery obtained for absorption maxima, first order derivative spectroscopy and area under the curve was found to be in the range of 92.21%, 99.09%, 98.48%. The results were shown in the table.3 % RSD and the precision results were presented in the table.4 satistical revolution revealed that relative standard deviation of drugs at different concentration levels for 6 times was less than 2.0 (intraday-0.177inter day-0.147). Hence, the proposed were validated in terms of linearity, precision, and accuracy. The present work provides an accurate and sensitive method for the analysis of Daurnavir in bulk and tablet formulation.

Table 2: Optical characteristics of the proposed methods

S.NO.	Parameter	Method A	Method B	Method C
1	Linearity(µg/ml)	5-40	5-40	5-40
2	Linearity eqution	y=0.022X+0.016	Y=0.414x-0.360	Y=0.021x+0.015
3	Slope±SD	0.022±0.0016	0.414±0.0015	0.021±0.0032
4	Intercept ±SD	0.016±0.0035	0.0015±0.032	0.0032±0.026
5	Correlation coefficient	0.997	0.995	0.997
6	LOD	390 ng	20 ng	403 ng
7	LOQ	1170 ng	60 ng	1210 ng

Table 3: Recovery studies of proposed methods

Method	Level of recovery	Pre analyzed conc(µg/ml)	Amount added(µg/ml)	Amount found(µg/ml)	%Recovery
Method A	50	5	2.5	6.909	92.12
	100	5	5	9.909	99.09
	150	5	7.5	12.31	98.48
Method B	50	5	2.5	6.549	86.667
	100	5	5	9.2442	92.44
	150	5	7.5	12..00	96.00
Method C	50	5	2.5	7.54	100.66
	100	5	5	9.41	94.16
	150	5	7.5	12.33	98.66

Table 4: Precision Studies of Proposed Methods

Intra day				Inter day		
Method	**Concentration(µg/ml)**	**Mean ±SD**	**%RSD**	**Concentration(µg/ml)**	**Mean ±SD**	**%RSD**
A	15	14.86±0.0046	0.137	15	14.44±0.0065	0.135
B	15	14.79±0.0072	0.165	15	14.37±0.0045	0.164
C	15	14.91±0.0015	0.231	15	14.29±0.0076	0.144

CONCLUSION

The three spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and %RSD calculated for the methods are within the limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence it can be conducted that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of Daurnavir bulk and formulation. The proposed methods were found to be simple, economical, eco-friendly, rapid, precise and accurate for the determination of Daurnavir in tablet dosage form. There is good scope for other poorly water soluble drugs which may be tried to get solubilized in 8M ureasolution (as hydrotropic agent) to carry out their spectrophotometer analysis excluding the use of costlier and unsafe organic solvents. Thus, it can be easily and conveniently adopted for routine quality control analysis.

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REFERENCE

1. Babu A, G. Raveendra Lakshmana Rao and J. Venkateswara Rao Development and Validation of Novel HPLC method for Estimation of Darunavir in Pharmaceutical Formulation Int J of Res in Pharmacy and Chemistry 2013;3(2).
2. Back D, Sekar V, Hoetelmans RM, Darunavir pharmacokinetics and drug interactions. International Journal of Pharmacy and PharmaceuticalSciences, 2008; 13(1): 1-13.
3. Skoog A and Donald West M. Applications of Ultraviolet and Visible absorption measurements. 2nd ed.Saunders College publishers; 1980. P.169-200.
4. Krishna Kumar Rao KVV 1, Phanindra B and Rajesh K, Spectrophotometric method forestimation of darunavir ethanolate by using MBTH reagent in bulk and pharmaceuticaldosage form. Pharm Analysis & Quality Assurance, 2013; 4.
5. Ghante M. R., Shelar R. S., Sawant S. D., Kadam M. M., Development and validationof UV spectrophotometric method for estimation of darunavir ethanolate in bulk and tabletdosage form, are described. International Journal of Pharmacy and PharmaceuticalSciences, 2014; 6(7).
6. Shinde VR, Gosavi SA, Pawar SS, Kasture VS, Musmade DS and Shinde VR. Development and validation of UV spectroscopic method for determination of Darunavir in bulk and tablet formulation. Inventi Rapid: Pharm Anal Qual Assur. 2013; 12:586.
7. Nageswara Rao R, Ramachandra B and Santhakumar K. RP-HPLC separation and characterization of unknown impurities of a novel HIVprotease inhibitor Darunavir by ESIMS and 2D NMR spectroscopy. J Pharm Biomed Anal. 2013; 75:186- 191.