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Simultaneous Estimation of Darunavir Ethanolate and Ritonavir in Combined Dosage Form

[*1] Hemlata Nimje, [2] Pranita Kore, [3] Sagar Wankhede, [4] Ayushi Gadekar

[1] Assistant Professor, Department of Pharmaceutical Chemistry, SPPU, JSPM Charak College of Pharmacy and Research, Wagholi, Pune, Maharahstra, India

[2] Research Scholar, Department of Pharmaceutical Chemistry, SPPU, JSPM Charak College of Pharmacy and Research, Wagholi, Pune, Maharahstra, India

[3] Principal and Professor, Department of Pharmaceutical Chemistry, SPPU, JSPM Charak College of Pharmacy and Research, Wagholi, Pune, Maharahstra, India

[4] Department of Pharmaceutical Chemistry, SPPU, JSPM Sawant College Pharmacy and Research, Hadapsar, Pune, Maharahstra, India

Abstract
Sensitive, spectrophotometric, simultaneous equation method (Method A) and Q-analysis method (Method B) have been developed for the simultaneous estimation of Darunavir ethanolate (DAR) and Ritonavir (RIT) in combined dosage form. The estimation by method A was carried out at the wavelength of 267.50 nm and 241.00 nm for DAR and RIT respectively. Method B involved the formation of absorbance equation at 244 nm (isoabsorptive point) and at 267.50 nm the maximum absorption of DAR. The linearity was found to be 8-35 µg/ml for DAR and 5-70 µg/ml for RIT by both methods. The recovery study of method a in tablet were found to be 102.99±1.22% and 101.79±1.46% for DAR and RIT respectively. The recovery result of DAR and RIT were found to be 101.93±0.79% and 99.04±0.98 for method B respectively. Both the methods were validated as per ICH guidelines.

Keywords: UV spectrophotometry, Darunavir ethanolate, Ritonavir, Simultaneous determination, Q-analysis method

1. Introduction
Darunavir a new protease inhibitor (PI), is used in the treatment of human immunodeficiency virus (HIV) type-1 infection. According to in-vitro experiments, it is active against HIV-1 with PI resistance mutations and against PI resistant clinical isolates [1]. Darunavir ethanolate (DAR) is chemically [(1*S*,2*R*)-3-[[(4-aminophenyl)sulfonyl] (2-methylpropyl)amino)-2-hydroxy-1-(phenylmethyl) propyl] carbamic acid (3*R*,3a*S*,6a-*R*)-hexahydrofuro [2,3-b] furan-3-yl ester monoethanolate [2]. Ritonavir (RIT) is a selective, competitive and reversible inhibitor of both HIV-1 and HIV-2 proteases, it is widely used in the treatment of AIDS and particularly to inhibit liver enzyme, viz., cytochrome P450-3A4 (CYP3A) [3]. Ritonavir is chemically (5*S*,8*S*,10*S*,11*S*)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo8,11bis (phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid-5-thiazolylmethyl ester [4]. The combination therapy of two drugs is effective for the treatment AIDS. Literature survey revealed that different analytical method for determination of DAR in dosage form and biological fluid viz. UV [5], HPLC [6, 7, 8, 9, 10], HPLC-MS [11, 12] and LC–ESI-MS [13]. The estimation of RIT in bulk, formulation and biological samples was also reported in literature viz. UV [14, 15, 16], HPLC [17, 18, 19, 20, 21], LC [22, 23], HPTLC [24] and LC-MS/MS [25, 26]. Liquid chromatography reported for analysis combination of Darunavir ethanolate and Ritonavir [27]. The present work describes an accurate, precise, specific and validated UV spectrophotometric method for simultaneous determination of these drugs.

2. Materials and Methods
2.1 Instrument and reagents
A Shimadzu UV-Visible Spectrophotometer 1800, with 1 cm matched quartz cell was used. Analytical grade chemicals were used. Darunavir ethanolate and Ritonavir drugs were obtained from Emcure Pharmaceuticals, Pune, Maharashtra, India.

2.2 Preparation of standard stock solution
Darunavir (10 mg) and Ritonavir (10 mg) were weighed separately and transferred in two different 100 ml volumetric flasks. Both the drugs were dissolved in 30 ml of methanol by shaking and volume was made up to the mark with methanol to obtain final concentration of 100 µg/ml of each component. The stock solutions were used in analysis. These dilutions were scanned in the wavelength range of 200-400 nm. Wavelengths Maxima of 267.50 nm and 241.00 nm were selected for DAR and RIT respectively as shown in Figure 1. From this stock solution, various standard solutions of DAR were prepared by pipetting 0.8-3.5 ml of stock solutions (100 µg/ml) of DAR in 10 ml of volumetric flasks and the volume was adjusted up to mark by methanol to obtain the concentrations of 8-35 µg/ml. Similarly standard solutions of RIT were prepared by pipetting 0.5-7 ml of stock solutions

100 µg/ml) of RIT in 10 ml of volumetric flasks and the volumetric flasks and volume was made up mark by methanol to obtain the final concentrations of 5-70 µg/ml.

2.3 Simultaneous equation Method A

Diluted standard solution of DAR (30 µg/ml) and RIT (5 µg/ml) were scanned over the range of 200-400 nm to select the maxima wavelength (λ_{max}). The λ_{max} of DAR 267.50 nm and RIT 241 nm were selected for analysis by simultaneous equation method. The overlay UV spectrum of DAR and RIT was given in figure 1. The linearity was determined and found to be in the range of 8-35 µg/ml and 5-70 µg/ml of DAR and RIT respectively. The optical characteristic and statistical data obtained from the regression equation of calibration curve were given in figure 3 and 4. Absorbance of these solutions were measured at selected wavelengths as A_1 and A_2 and concentrations of two drugs in each sample were calculated by using Cramer's rule and matrices and following equations [28].

$$Cx = A_2\, ay_1 - A_1\, ay_2 / ax_2\, ay_1 - ax_1\, ay_2$$
$$Cy = A_1\, ax_2 - A_2\, ax_1 / ax_2\, ay_1 - ax_1\, ay_2 \ldots\ldots\ldots [1]$$

Where C_X and C_Y were concentration of RIT and DAR, A_1 and A_2 absorbance of RIT and DAR solution at 241.00nm and 267.50 nm respectively, a_{x1} and a_{x2} are absorptivity of RIT at 241.00nm and 267.50 nm, a_{y1} and a_{y2} were absorptivity of DAR at 241.00 and 267.50 nm respectively.

2.4 Absorption ratio method or Q – analysis method B

Two wavelengths were selected at 244.00 nm as isoabsorptive point for both drugs and another absorption maxima wavelength 267.50 nm (figure 2). The absorbance of the standard and sample solutions were prepared and measured as per method A. The linearity was determined and found to be in the range of 5-70 µg/ml and 8-35 µg/ml of RIT and DAR respectively. The absorptivity values for both standard drugs at the selected wavelength as per simultaneous equation method were employed for determination of Q-analysis method and at isoabsorptivity point for combination. The concentrations of drugs in sample solution were determined by using the following formula [28].

$$Cx = [(Qm-Qy)/(Qx-Qy)] \times [A/Ax_1] ;$$
$$Cy = [(Qm-Qx)/(Qy-Qx)] \times [A/Ay_1] \ldots\ldots [2]$$

Where Cx and Cy were concentration of RIT and DAR in g/100 ml, Qm is ratio of absorbance of laboratory mixture at 244 nm and 267.50 nm, Qx and Qy were Ratio of absorbance of RIT and DAR at 244 nm and 267.50 nm, Ax_1 is absorptivity of RIT at 244 nm and Ay_1 is absorptivity of DAR at 267.50 nm.

2.5 Assay of pharmaceutical formulation

Ten tablets were used. The tablets were weighed and totally powdered. The mass equivalent to one tablet DAR and RIT content was weighed and transferred into 100 ml volumetric flask and dissolved in methanol by sonication for 10 mins. 0.5 ml of this solution was transferred to 10 ml volumetric flask and volume was made up to mark with methanol to obtain final concentration of 5 µg/ml of RIT and 30 µg/ml of DAR. The solution was filtered through Whitman filter paper and used for the analysis. The solutions were scanned in the UV spectrophotometer at the selected wavelengths and values of absorbances and absorbtivity were recorded. The % amount found was determined by using equation 1 for simultaneous method A and equation 2 for Q-Analysis method B. The three determinations were performed.

3. Validation

The methods were validated according to ICH guidelines [ICH Q2B] to determine the specificity, linearity, accuracy, precision and ruggedness.

3.1 Specificity

The specificity of both the methods was checked for any interference of excipients in the analysis of drug solution under optimized conditions. The interference were observed for any change in the absorbance and λ_{max} of both drugs.

3.2 Linearity

Each concentration of working solution of DAR and RIT were observed under UV system. The absorbance was determined for each concentration of the DAR and RIT independently. The calibration curves were obtained with linear response of DAR and RIT in the concentration ranges of 8-35 µg/ml and 5-70 µg/ml.

3.3 Accuracy

The accuracy of methods was determined by carrying out recovery studies at three different levels (80, 100 and 120) on the basis of the label claim. At each level, three determinations were performed simultaneously and percentage recovery and RSD was calculated to prove the accuracy of the methods. Data of accuracy study is given in table 3.

3.4 Precision

The precision of the method was established by carrying out triplicate determination of concentration of DAR and RIT by repeatability (intraday) and intermediate precision (interday). The intraday precision was performed on the same day on same sample at particular time interval. The interday precision was performed on the three consecutive days on same sample solution. The results were obtained and percentage RSD was calculated in order to establish the precision of the method. Data of precision study is given table 3.

3.5 Ruggedness and robustness

The ruggedness of both the methods were performed by changing analysts and instruments. The results are given in table 4. The robustness was performed by changing solution composition and wavelength for both methods (table 5).

4. Results and Discussion

The linearity was observed over the different concentration of DAR and RIT by exposing the solution to UV at λ_{max} 267.50nm and 241 nm respectively (Table 1). Both the methods were found to be specific as there is no change in the λ_{max} and absorbance of DAR and RIT in the presence of the excipients. The mean percentage recoveries of tablet containing DAR and RIT were in the range of 98.24 to

102.54% with %RSD less than 2, indicating the suitability of the developed method in quantifying the concentration of DAR and RIT in tablet dosage form (Table 2). The accuracy of the method was confirmed by determining the average recoveries from the samples by applying the standard addition method. Repeatability (intra-day precision), intermediate (interday) precision (table 3) of the analytical method was found to be reliable based on %RSD (<2%).

5. Tables and Figures

Table 1: Statistical parameters for DAR and RIT

Parameters	Method A		Method B	
	DAR	RIT	DAR	RIT
Linearity range (µg/ml)	8-35	5-70	8-35	5-70
Regression coefficient (r^2)	0.999	0.999	0.999	0.998
Slope	0.038	0.019	0.038	0.019
Intercept	0.014	0.036	0.014	0.016

Table 2: Assay of tablet formulations by UV method

Methods →	Method A			Method B		
Components →	Label claim (mg)	% Amount found*	%RSD	Label claim (mg)	% Amount found*	%RSD
DAR	150	102.99	1.22	150	101.93	0.79
RIT	25	101.79	1.46	25	99.04	0.98

N*=3

Table 3: Accuracy and precision study for DAR and RIT

Sample	% Level	Method A				Method B			
		Intraday		Interday		Intraday		Interday	
		Mean*	%RSD	Mean*	%RSD	Mean*	%RSD	Mean*	%RSD
DAR	80	100.25	0.75	99.97	0.71	98.99	0.62	98.37	0.21
	100	102.54	0.70	99.03	0.65	98.26	0.35	98.27	0.19
	120	101.02	0.52	100.25	0.53	98.56	0.24	98.65	0.36
RIT	80	99.92	0.89	98.65	0.42	99.89	0.51	99.52	0.25
	100	99.97	0.92	98.24	0.25	99.35	0.32	99.01	0.12
	120	99.89	0.87	98.52	0.36	99.52	0.39	99.36	0.27

N*=3

Table 4: Ruggedness study of DAR and RIT

Sr. No.	Type of analysis	%Estimated*±%RSD	
		Method A	Method B
1	Analyst-1	100.58±0.990	99.69±0.590
2	Analyst-2	99.42±0.588	98.97±0.101
3	Instrument-1	100.96±0.993	100.15±0.998
4	Instrument-2	98.36±0.968	98.06±0.100

*N=5

Table 5: Robustness study of UV spectrophotometric method

Parameter	%Estimated*±%RSD	
	Method A	Method B
Solvents		
Methanol	99.65±0.980	98.55±0.583
Acetonitirle	100.25±0.986	99.23±0.587
Wavelength (±1 nm)		
266.50 (DAR), 240 (RIT)	100.36±0.987	99.96±0.996
268.50 (DAR), 243 (RIT)	101.88±0.990	101.80±0.880

*N=5

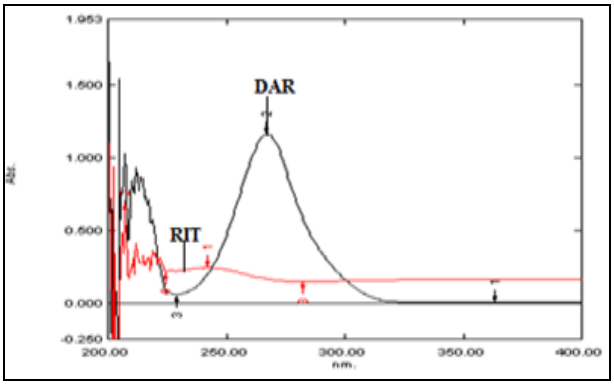

Fig 1: UV spectrum of DAR and RIT for Simultaneous equation method A

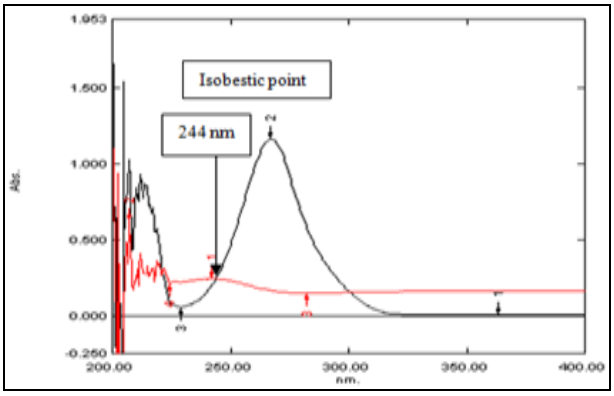

Fig 2: UV absorption spectrum of DAR and RIT for Q-analysis method B

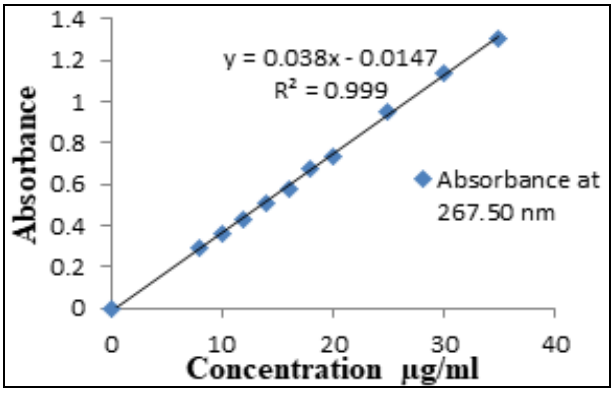

Fig 3: Calibration curves for DAR by Simultaneous equation method

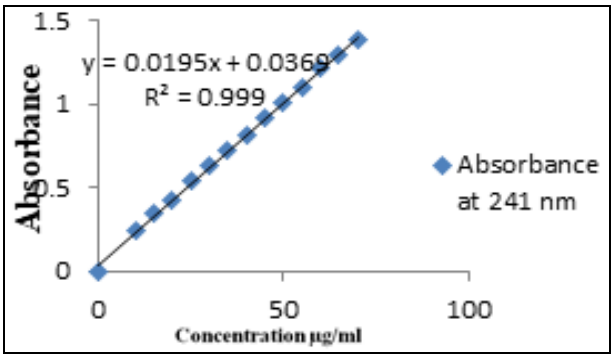

Fig 4: Calibration curves for RIT by Simultaneous equation method

6. Conclusion

The proposed two spectrophotometric methods were found to be simple, accurate, economical, reproducible and rapid can be employed for routine analysis of formulations.

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