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Development and Validation of UV Spectroscopic Methods for Simultaneous Estimation of Darunavir and Rilpivirine HCl in Tablet Formulation

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ABSTRACT

Two simple, accurate, precise, reproducible and economical UV spectroscopic methods (A & B) for simultaneous estimation of Darunavir and Rilpivirine HCl in tablet dosage form have been developed. Method A employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 265 nm and 290 nm which are the λ_{max} values of Darunavir and Rilpivirine respectively. Darunavir and Rilpivirine HCl show linearity at all the selected wavelengths and obey Beer's law in the concentration range of 10-35 µg/mL and 10-80 µg/mL respectively. Recovery studies for Darunavir and Rilpivirine HCl were performed and the percentage recovery for both the drugs was obtained in the range of 98.1-99.7% (Method A) and 98.0-100.4% (Method B) confirming the accuracy of the proposed method. Both the methods showed good reproducibility and recovery with %RSD less than 2. Statistical validation of the data shows that the proposed methods can be successfully applied for the routine analysis of drugs in commercial tablets.

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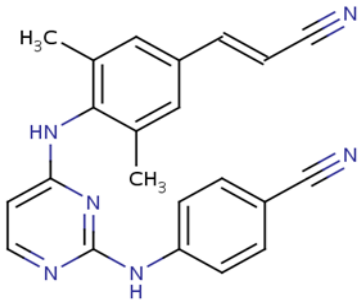
INTRODUCTION

Darunavir (DRV), sold under the brand name Prezista among others, is an antiretroviral medication used to treat and prevent HIV/AIDS. It is generally recommended for use with other antiretrovirals. It is often used with low doses of ritonavir or cobicistat to increase darunavir levels. It may be used for prevention after a needle stick injury or other potential exposure. It is taken by mouth once to twice a day.[1] Common side effects include diarrhea, nausea, abdominal pain, headache, and rash. Severe side effects include allergic reactions, liver problems, and skin rashes such as toxic epidermal necrolysis.[1] While poorly studied in pregnancy it appears to be safe for the baby.[2] It is the protease inhibitor (PI) class and works by blocking HIV protease.[1] Rilpivirine (TMC278, trade name Edurant) is a pharmaceutical drug, developed by Tibotec, for the treatment of HIV infection.[1][2] It is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with higher potency, longer half-life and reduced side-effect profile compared with older NNRTIs, such as efavirenz.[3][4] Rilpivirine entered phase III clinical trials in April 2008,[5][6] and was approved for use in the United States in May 2011.[7] A fixed-dose drug combining rilpivirine with emtricitabine and tenofovir was approved by the U.S. Food and Drug Administration in August 2011 under the brand name Complera;[8] it was licensed in the European Union under the brand name Eviplera in November 2011.[9]

Literature survey revealed that various analytical methods such as UV spectroscopy (Bombale *et al.*, 1997*;* Sharma *et al.*, 2011), HPLC (Bhatia *et al*., 1999), pulse polarography (Salvi and Sathe, 2010) have been reported for the simultaneous estimation of both the drugs. This study is useful because these two drugs are commonly administered simultaneously. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability, and ease of use (Nijhu *et al.*, 2011). The aim of the present investigation is to develop a simple, sensitive and reproducible UV Spectrophotometric method for analysis of Darunavir and Rilpivirine HCl in a combined tablet dosage form and hence an economical method was developed and validated according to the ICH guidelines.

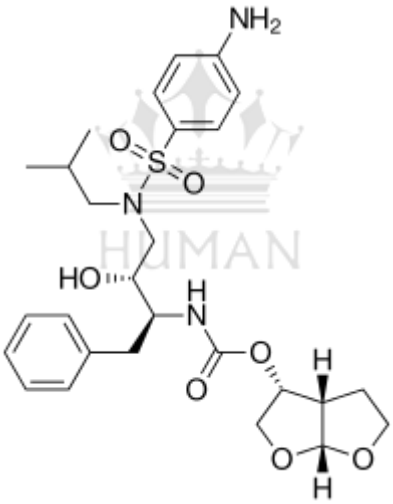
The structures of the drugs Darunavir and Rilpivirine Hydrochloride as shown below.



Structure of Rilpivirine Hydrochloride

IUPAC Name: 4-[[4-[[4-[(E)-2-cyanoethenyl]-2, 6-dimethylphenyl] amino-2-pyrimidinyl] amino] benzonitrile monohydrochloride. (Molecular weight: 402.886 g/mol)(Molecular formula: $C_{22}H_{19}ClN_6$)

Structure of Darunavir



IUPAC Name: [(1*R*,5*S*,6*R*)-2,8-dioxabicyclo[3.3.0]oct-6-yl] *N*-[(2*S*,3*R*)-4- [(4-aminophenyl)sulfonyl- (2-methylpropyl)amino]-3-hydroxy-1-phenyl- butan-2-yl] carbamate (Molecular Mass: 547.665 g/mol) (Molecular Formula: $C_{27}H_{37}N_3O_7S$)

MATERIALS AND METHODS

Instruments

Absorbance measurements were made on Shimadzu 1800 UV/Visible spectrophotometer with a pair of 10 mm matched quartz cells, Shimadzu digital balance for weighing and Cintex sonicator were used.

Chemicals and reagents

All chemicals were of analytical reagent grade and solutions were prepared with double distilled water. Darunavir and Rilpivirine HCl gift samples were obtained from Dr. Reddy's Laboratories, Hyderabad. Potassium dihydrogen orthophosphate and Methanol were procured from E. Merck Co., Mumbai, India. Sodium hydroxide was purchased from Qualigen's. Combined tablets of. Darunavir and Rilpivirine HCl were procured from the local pharmacy.

Procedure

Preparation of phosphate buffer (pH 6.8)

Accurately weigh about 0.896 gm of NaOH, 6.794 gm of KH_2PO_4, dissolve in distilled water and make up the volume to 1 liter with distilled water.

Preparation of stock solution (1000 µg/mL)

Accurately weighed the quantity of pure Darunavir (10 mg) and pure Rilpivirine HCl (10 mg) were transferred into two separate 10 ml volumetric flasks, dissolved in methanol and made up the volume to 10 ml with the same solvent. The stock solution was sonicated for 5min.

Preparation of working standard solution (100 µg/mL)

From the above stock solution, 1 ml each of Darunavir and Rilpivirine HCl were taken, transferred to separate 10 ml volumetric flasks and the volume was made up to 10 ml with phosphate buffer.

Simultaneous Equations Method (Method A)

10 µg/mL solutions of Darunavir and Rilpivirine HCl were prepared separately in phosphate buffer (pH 6.7) and the solutions were scanned against blank in the entire UV range to determine the λ_{max} values. Clear peaks were observed at 262 nm for Darunavir and 290 nm for Rilpivirine. Hence these wavelengths were chosen as the λ_{max} values for each drug respectively (Fig. 1). Standard solutions of Darunavir and Rilpivirine in the concentration range of 10-35 µg/mL and 10-80 µg/mL respectively were prepared in phosphate buffer and the absorbance of these solutions was measured at 262 nm and 290 nm. Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using

these absorptivity values, A (1%, 1cm).Where C_x and C_y are the concentrations of Darunavir and Rilpivirine HCl measured in gm/100 ml in sample solutions. A_1 and A_2 are the absorbances of a mixture at selected wavelengths 262 nm and 290 nm respectively.

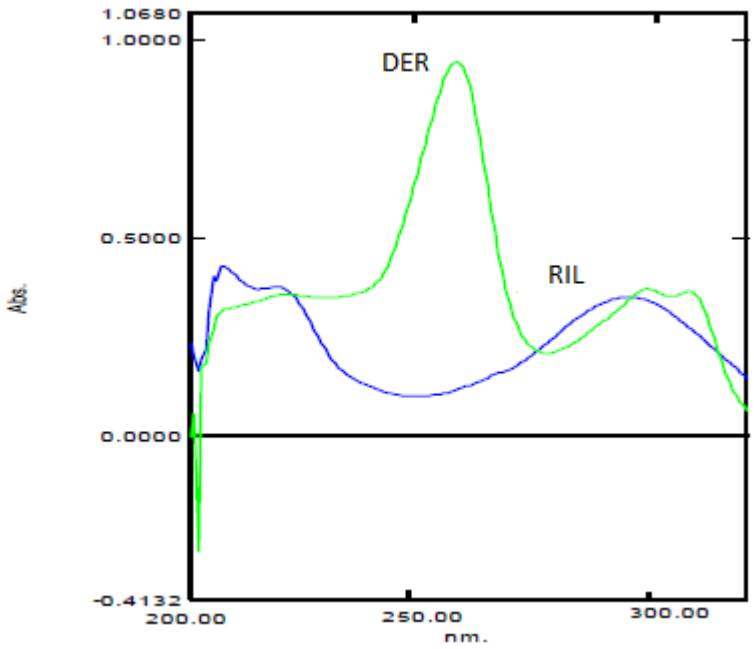


Fig. 1: Overlay spectra of Darunavir (DER) and Rilpivirine HCl(RIL)

Absorbance Ratio Method/ Q-Analysis (Method B)

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. E.g. two dilutions of the same substance give the same absorbance ratio A_1 / A_2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances are measured at two wavelengths, one being the λ_{max} of one of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), i.e., an iso-absorptive point (Beckett and Stenlake, 2005). A series of standard solutions of Darunavir and Rilpivirine HCl in the concentration range of 10-35 µg/mL and 10-80 µg/mL respectively were prepared in phosphate buffer and the absorbance of these solutions was measured at 262 nm (iso-absorptive point) and 290 nm (λ_{max} of Darunavir) (Fig. 1).Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. The absorptivity values are reported in Table 1.

Table 1: Absorptivity values (A 1%, 1 cm) of Darunavir and Rilpivirine HCl for Methods A & B.

	Absorptivity , A (1%, 1cm)							
Conc(µg/mL)	**Method A**				**Method B**			
	Darunavir		**Rilpivirine HCl**		**Darunavir**		**Rilpivirine HCl**	
	262 nm	**290 nm**	**262 nm**	**290 nm**	**292 nm**	**262 nm**	**292 nm**	**262 nm**
10	944.6	340.5	120	372.5	217	943.6	217	120
15	956.67	339.87	-	-	201	955.66	-	-
20	926.05	335.45	113.6	360.8	203.15	926.05	200.5	113.6
25	910.04	330.6	-	-	201.32	910.04	-	-
30	905.1	341.67	-	-	202.6	905.1	-	-
35	899.31	343.51	-	-	202.4	899.31	-	-
40	-	-	103.65	368.25	-	-	204.53	103.6
60	-	-	103.18	374.5	-	-	198.5	103.1
80	-	-	98.6	360.39	-	-	192.5	98.6
Mean	923.63	338.59	107.81	367.29	204.5783	924.62	202.605	107.81

The concentration of two drugs in the mixture was calculated by using the following equations:

$$C_X = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a_{X1}}$$

$$C_Y = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{a_{Y1}}$$

Where, A_1 and A_2 are the absorbances of mixture at 290 nm and 262 nm,a_{x1} (107.8), a_{x2} (367.3) and a_{y1} (924.6), a_{y2} (348.6) are A (1%, 1 cm) of TNZ and CPX at 292 nm and 271 nm respectively, $Q_m = A_2\ A_1$, $Q_x = a_{x2}\ a_{x1}$, and $Q_y = a_{y2}\ a_{y1}$

Assay of tablets by Method A and B

20 commercial tablets of DRV and RIL were triturated and powder equivalent to 10 mg of RIL and 8.0 mg of DRV respectively was weighed and transferred to 10 ml volumetric flask, dissolved in methanol, volume adjusted up to the mark with the same solvent and mixed well with the help of a sonicator. The solution was filtered through Whatman filter paper no 40.1 ml of the above filtrate was diluted to 10 mL with phosphate buffer to obtain a 100 µg/mL solution with respect to RIL. From this solution, an aliquot was taken and made up the volume to 10 mL with phosphate buffer expected to contain 10 and 8 µg/mL of DRV and RIL respectively. The absorbance of the sample solution was measured at 262 nm and 290 nm(Method A), 290 nm and 262 nm (Method B) and the data analyzed accordingly using the necessary equations. The analysis procedure was repeated for 6 times with tablet formulations. The result of analysis of tablet formulation is reported in Table 2**.**

Table 2: Results of simultaneous estimation of marketed formulation (Darunavir and Rilpivirine HCl) for Methods A & B.

Method	**Label claim (mg/tablet)**		***Amount obtained (mg/tablet)**		***Recovery(%) ± SD**	
	DRV	**RIL**	**DRV**	**RIL**	**DRV**	**RIL**
Method A	500	600	494.5	598	98.9±0.25	99.8±0.27
Method B	500	600	496	602.4	99.4±0.21	100.4±0.18

*Mean of six estimations; DRV = Darunavir; RIL= Rilpivirine

Validation (Method A & B)

Linearity

Appropriate dilutions of working standard solutions for DRV and RIL were prepared in the concentration range of 10-35 µg/mL and 10-80 µg/mL, respectively and analyzed as per the developed methods A & B. The results are reported in Table 3.

Table 3: Regression analysis of calibration curves and summary of validation parameters for Methods A & B.

Sl.No.	Parameter	Drug	Method A		Method B	
			262 nm	290 nm	262 nm	290 nm
1	Beer's law limit (µg ml^{-1})	Darunavir	10-35			
		Rilpivirine HCL	10-80			
2	Molar absorptivity (l mol^{-1} cm^{-1})	Darunavir	30614	11223	6779	30614
		Rilpivirine HCL	2666	9082	5010	2666
3	Sandell's sensitivity (µg/cm^2/0.001)	Darunavir	0.01	0.029	0.046	0.01
		Rilpivirine HCL	0.083	0.026	0.05	0.91
4	Intercept(c)	Darunavir	0.0418	0.004	0.004	0.041
		Rilpivirine HCL	0.018	0.0071	0.018	0.018
5	Slope (m)	Darunavir	0.0895	0.034	0.020	0.089
		Rilpivirine HCL	0.09	0.0365	0.019	0.009
6	Correlation coefficient (r^2)	Darunavir	0.9992	0.999	0.999	0.999
		Rilpivirine HCL	0.998	0.9993	0.999	0.9989

Accuracy and Recovery Studies

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels according to ICH guidelines. A series of solutions of DRV and RIL at 80%, 100%, and 120% of the standard preparation in the ratio of the formulation were prepared and checked for accuracy by determining the absorbance values at λ_{max} of 262 nm and 290 nm (Method A) 290 nm and 262 nm (method B) respectively. To a fixed concentration of the formulation, varying concentrations of pure drug solutions were added and percentage recoveries calculated. The result of the analysis is given in Table 4.

Table 4: Results for recovery studies.

Level of Recovery (%)	Drug in tablet (µg)		Drug added (µg)		*Drug recovered (µg)				*Recovery(%) ± SD			
					Method A		Method B		Method A		Method B	
	DRV	**RIL**	**DRV**	**RIL**	**DRV**	**RIL**	**DRV**	**RIL**	**DRV**	**RIL**	**DRV**	**RIL**
80	8	10	6.4	8	14.1	17.8	13.96	17.7	98.8±0.17	99±0.13	98±0.5	98.9±1.3
100	8	10	8	10	15.7	19.8	15.6	19.5	98.9±0.18	98.7±0.26	98.7±0.15	99.7±0.19
120	8	10	9.6	12	17.3	21.6	17.2	21.5	98.1±0.42	98.3±0.45	98.3±0.28	98.3±1.01

*Mean of three estimations; DRV = Darunavir; RIL= Rilpivirine

Precision

Precision studies were performed at three different concentrations in the ratio of the formulation, each concentration prepared three times for DRV and RIL together. The result of the analysis is given in Table 5.

Table 5: Results for precision studies.

Sr. No.	**Conc. (µg/mL)**		***Assay(%) ± SD**				***RSD (%)**			
			Method A		**Method B**		**Method A**		**Method B**	
	DRV	RIL	DRV	RIL	DRV	RIL	DRV	RIL	DRV	RIL
1	8	10	98.7±0.23	98.93±0.12	98.26±0.64	99.3±0.41	0.26	0.12	0.65	0.41
2	16	20	98.73±0.23	99.26±0.23	99.2±0.69	98.7±0.26	0.24	0.23	0.71	0.26
3	32	40	98.83±0.57	98.38±0.68	98.2±0.69	98.67±0.29	0.59	0.69	0.75	0.29

*Mean of three estimations; DRV = Darunavir; RIL= Rilpivirine

RESULTS AND DISCUSSION

DRV and RIL exhibited maximum absorption at 262 nm and 290 nm (Method A), they were also analyzed at 290 nm and 262 nm (Method B). DRV obeyed Beer's law in the concentration range of 10-35 µg/mL while RIL obeyed the Beer's law in the concentration range of 10-80 µg/mL (Method A & B). The precision data shows that the reproducibility of the assay procedure was satisfactory. The recovery studies done by standard addition method has given satisfactory results with an average percentage recovery of 98.6% and 98.7% (Method A), 98.4% and 99.0% (Method B) for DRV and RIL respectively. The regression analysis of the calibration curves and the optical characteristics such as Beer's law limits, molar absorptivities, and Sandell's sensitivities were also determined. The results are shown in Table 3.

CONCLUSION

Two new, simple, sensitive and economical UV spectrophotometric methods were developed for the simultaneous analysis of Darunavir and Rilpivirine in bulk and in pharmaceutical formulations. The developed methods were validated and from the statistical data, it was found that the methods were linear, accurate and precise and can be successfully applied for the analysis of pharmaceutical formulations without the interference of excipients.

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