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A NOVEL COST EFFECTIVE SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF RITONAVIR AND LOPINAVIR FROM COMBINED TABLET DOSAGE FORM

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ABSTRACT
A simple, cost effective and sensitive UV spectrophotometric method has been developed for the simultaneous estimation of Lopinavir (LOPI) and Ritonavir (RITO) in combined tablet dosage form. The method was validated as per ICH guidelines. Ritonavir and lopinavir exhibited maximum absorbance at 240nm and 260nm. Ritonavir obeyed Beer's law in the concentration range of 10-50µg/mL while Lopinavir obeyed the Beer's law in the concentration range of 100-500µg/mL. Molar absorptivity of the drugs were determined. The method was developed by using simultaneous equation method. Assay of the tablet were carried out and percentage of Ritonavir present in tablet was found to be 102.93%. The percentage of lopinavir present in tablet was found to be 102.08%. The amount of drug present in the tablet lies within the IP limit.

Keywords: UV Spectrophotometry, Ritonavir, Lopinavir, Simultaneous Equation.

INTRODUCTION

Ritonavir and Lopinavir are drugs belonging to the class of Protease inhibitors. These are the drugs used for the treatment of HIV infection. Ritonavir inhibits CYP3A4 and causes a number of drug interactions. It inhibits both HIV-1 and HIV-2 proteases. Ritonavir is usually administered along with low dose of other protease inhibitors. Ritonavir will inhibit the metabolism of co-administered protease inhibitors and increases their bioavailability and half-life. Hence dose and frequency of co-administered Protease inhibitors can be reduced. By this number of tablets of Protease inhibitors to be taken can be reduced. Ritonavir in combination with Lopinavir is widely administered drug combination for the treatment of HIV infection. Day by day the number of HIV infected case were increased in the world. Anti-retroviral therapy is preferred in order to treat HIV infection. Lopinavir and Ritonavir were one of the important drug combinations for the treatment of HIV infection. This drug combination played an important role

during the covid pandemic. Since it's an anti-viral drug this drug combination was also used in the initial stage of covid pandemic. Several analytical methods were developed for the estimation of these drugs from different pharmaceutical dosage forms. The existing analytical methods for the estimation of Lopinavir and Ritonavir in dosage forms requires expensive reagents.

Lopinavir RS and Ritonavir RS were insoluble water and soluble only in organic solvents. The existing spectrophotometric methods uses expensive and toxic solvents. Thus, the novel developed method uses less expensive solvents. The solvents used in this method belongs to class 3 solvent category in Residual solvents. The purpose of this work is to develop an analytical method which requires less consumption of organic solvents. This can reduce the cost and hence a simple spectrophotometric method can be developed which can used for the routine analysis of this drug combination.

MATERIALS AND METHODS

Collection of Materials:

Lopinavir RS and Ritonavir RS were procured from Yarrow chemicals Mumbai. Isopropanol HPLC grade procured from Merck specialities (P) Ltd Mumbai and Distilled water were used. Commercially available pharmaceutical dosage form - Emeltra Tablet (200mg Lopinavir IP & 50 mg Ritonavir IP) manufactured by Emcure Pharmaceuticals Ltd were utilised. Spectrometric studies were carried out by using HITACHI UV – Visible Spectrophotometer UH 5300. Shimadzu analytical balance ATX224 were used for weighing the drug samples.GT sonic Ultra sonic cleaner was used for dissolving the samples.

Selection of Solvent

Solvent was selected after assessing solubility of both drugs in different solvents. Both drugs were soluble in isopropyl alcohol. Isopropyl alcohol (IPA) is a least expensive organic solvents which belongs to class 3 in Residual solvents. Isopropyl alcohol is commonly used solvent as disinfectant. If the whole method is developed using IPA, then organic solvent consumption will be more. To reduce the organic solvent consumption, it is mixed with water. Hence both drugs were soluble in a mixture of IPA: Water in the ratio of 50: 50.

Determination of λ_{max}

Weighed accurately 25mg of Lopinavir RS and dissolved in 10 mL of IPA-Water mixture. Then diluted to 25mL with IPA-Water mixture. A 1000µg/mL solution were obtained. Pipette 1mL of the above solution and make up to 10mL with IPA-Water mixture to obtain 100µg/mL solution. λ_{max} of the above solution were scanned. The wavelength of maximum absorption was found to be at 260nm.

Weighed accurately 25mg of Ritonavir RS and dissolved in 10 mL of IPA-Water mixture. Then diluted to 25mL with IPA-Water mixture. A 1000µg/mL solution were obtained. Pipette 0.1mL of the above solution and make up to 10mL with IPA-Water mixture to obtain 10µg/mL solution. λ_{max} of the above solution were scanned. The wavelength of maximum absorption was found to be at 240nm.

Preparation of Calibration Curve

Weighed accurately 25mg of Lopinavir RS and dissolved in 10 mL of IPA-Water mixture. Then diluted to 25mL with IPA-Water mixture. A 1000µg/mL solution were obtained. Pipette out 1,2 ,3, 4 and 5mL from the above solution and make up to 10mL with IPA-Water mixture to obtain 100,200,300, 400 and 500µg/mL solution.

Weighed accurately 25mg of Ritonavir RS and dissolved in 10 mL of IPA-Water mixture. Then diluted to 25mL with IPA-Water mixture. A 1000µg/mL solution were obtained. Pipette out 0.1,0.2,0.3,0.4, and 0.5mL of the above solution and make up to 10mL with IPA-Water mixture to obtain 10,20,30,40 and 50µg/mL solution.

Assay

Preparation of Standard Solution

Weighed accurately 50mg of Lopinavir RS and 12.5mg of Ritonavir RS. Transferred into 50mL volumetric flask and dissolved in 25mL IPA-Water mixture. Sonicate for 5 minutes and makeup to 50mL.1mL of the above solution were diluted to 10mL.The resulting solution contains 100 µg/mL Lopinavir and 25 µg/mL Ritonavir.

Analysis of Tablet Formulation

Twenty tablets were finely powdered and weighed the sample of powdered tablets equivalent to Lopinavir (50 mg) and Ritonavir (12.5 mg) were transferred to a 50 mL volumetric flask and dissolved in IPA-Water mixture (50:50). The solution was sonicated for 30 min to ensure complete solubility of drug. The contents were made up to the mark with diluent and filtered through a Whatman filter paper. From the above solution 1 mL were transferred into 10mL volumetric flask. The resulting solution contains 100 µg/mL Lopinavir and 25 µg/mL Ritonavir. Absorbance of the resulting solution were measured at 240 and 260nm.

Method Validation

Accuracy

Accuracy was carried out by recovery studies. Recovery studies were carried out by applying the method to drug content present in tablet dosage form to which known amount of the reference standard of

Lopinavir and Ritonavir were added at 80%, 100% and 120% levels. The solution for recovery studies at 80%, 100% and 120% levels were prepared according to addition method. The solutions were filtered through Whatman filter paper and analysed by UV-Spectrophotometric method. At each level of recovery three determinations were performed.

Precision

A) Repeatability

The prepared tablet solution was analysed by repeatedly measuring absorbance at 240 and 260nm.

B) Intermediate precision

The intermediate precision was determined by measuring six replicate absorbance of the prepared sample solutions. The inter-day precision was obtained by measuring absorbance of six sample sets on different days.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were separately determined based on the standard deviation of the response of the calibration curve. The standard deviation of the y intercept and slope of calibration curve was used to calculate the LOD and LOQ.

LOD calculated from the formula, **LOD = 3.3*SD / S**

LOQ calculated from the formula, **LOQ=10 * SD / S**

Where, SD = Standard deviation, S = Slope

RESULT AND DISCUSSION

A simple, cost-effective UV spectrophotometric method has been developed for the simultaneous estimation of Lopinavir (LOPI) and Ritonavir (RITO) in combined tablet dosage form. Ritonavir and lopinavir exhibited maximum absorbance at 240nm and 260nm. Ritonavir obeyed Beer's law in the concentration range of 10-50µg/mL while Lopinavir obeyed the Beer's law in the concentration range of 100-500 µg/mL. Molar absorptivity of the drugs were determined. The method was developed by using simultaneous equation method. Assay of the tablet were carried out and percentage of Ritonavir present in tablet was found to be 102.93%. The percentage of lopinavir present in tablet was found to be 102.08%. The amount of drug present in the tablet lies within the IP limit. Thus, the developed method requires inexpensive and less toxic solvents. There is no spectrometric method for the determination this drug

combination in IP. Hence the developed method can be used for the determination Lopinavir and Ritonavir from tablet dosage form.

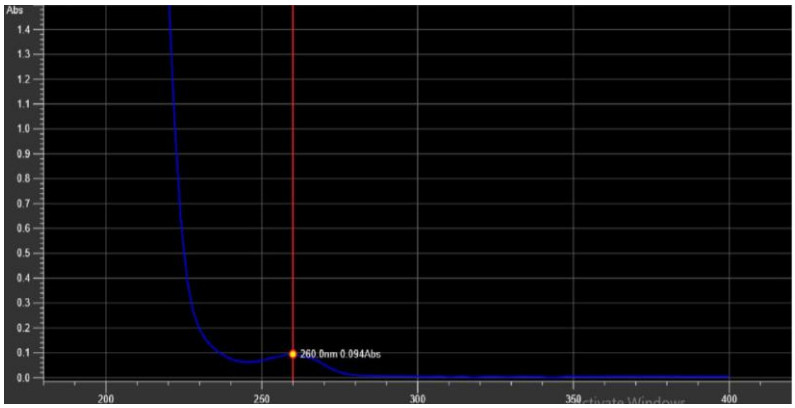

Fig 3: Zero order spectrum of Lopinavir

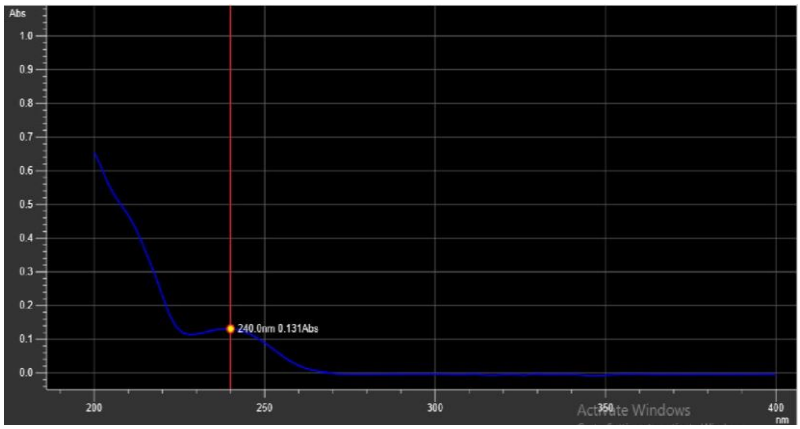

Fig 4: Zero order spectrum of Ritonavir

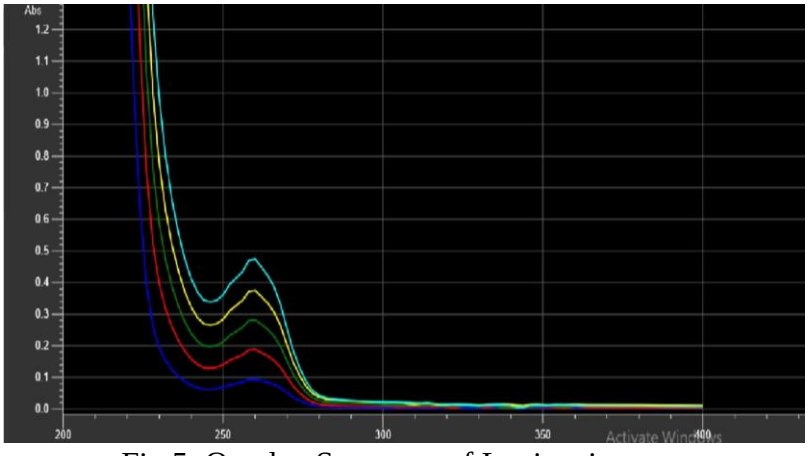

Fig 5: Overlay Spectrum of Lopinavir

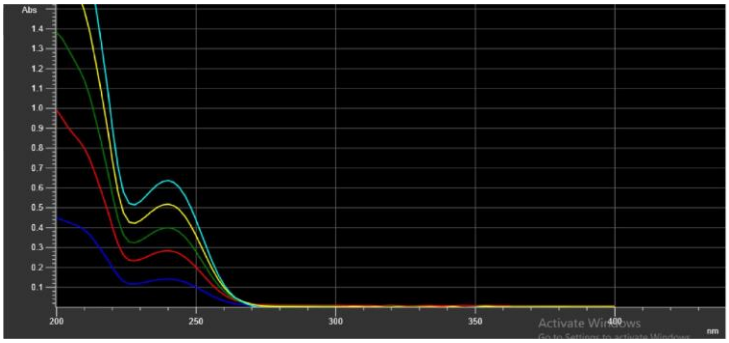

Fig 6: Overlay Spectrum of Ritonavir

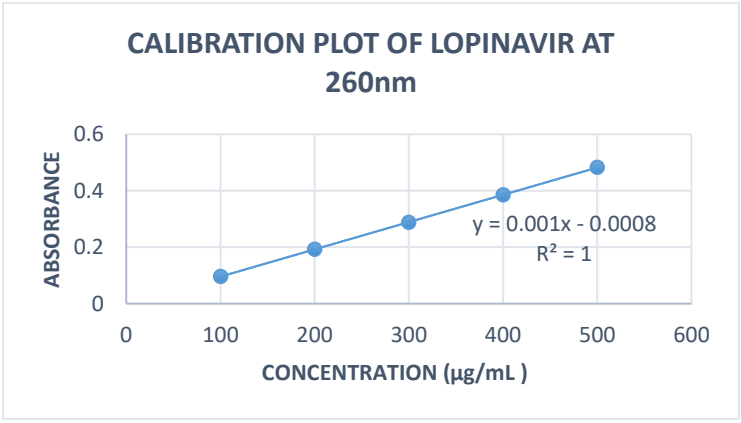

Fig 7: Calibration curve of Lopinavir at 260nm

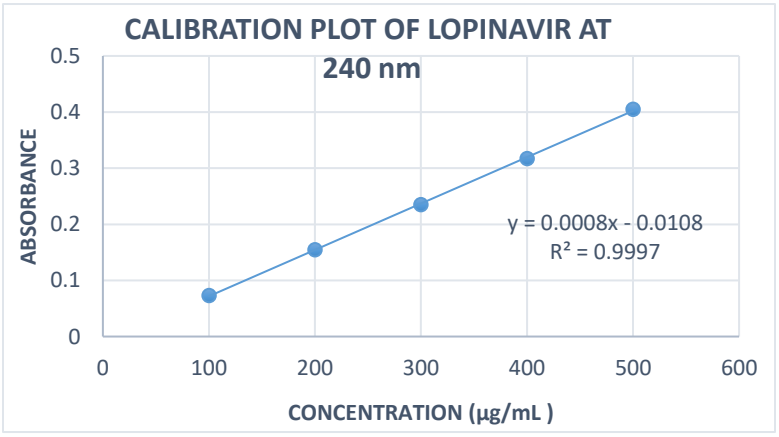

Fig 8: Calibration curve of Lopinavir at 240nm

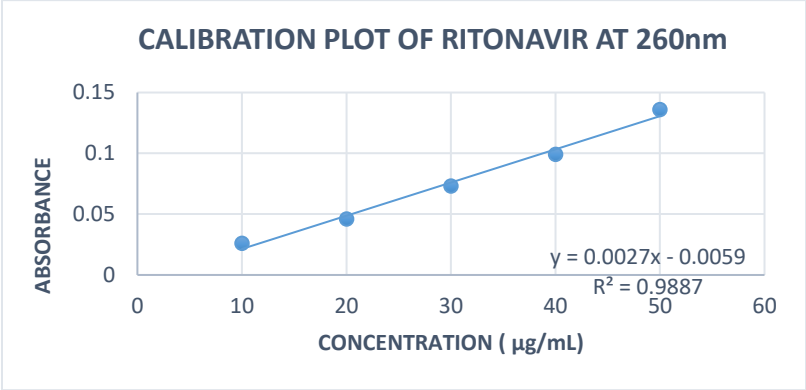

Fig 9: Calibration curve of Ritonavir at 260nm

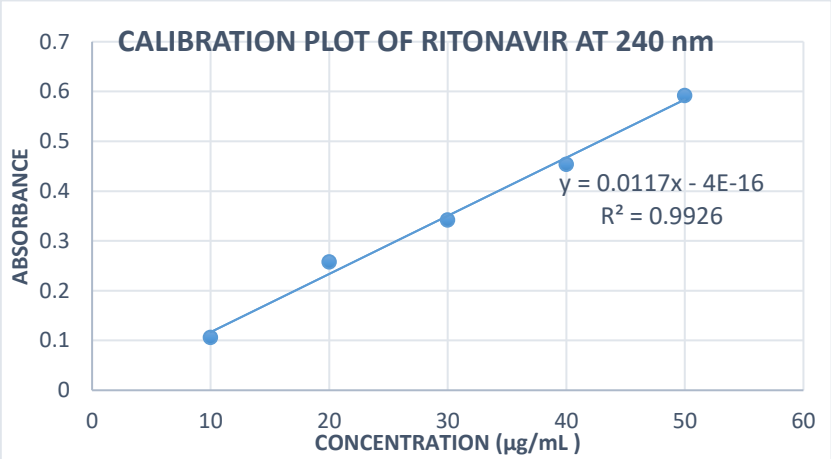

Fig 10: Calibration curve of Ritonavir at 240 nm

Table 1. Absorbance of Marketed Formulation

Sl No	Absorbance	
	240nm	260nm
1	0.378	0.161
2	0.379	0.162
3	0.380	0.163
4	0.378	0.162
5	0.379	0.162
6	0.377	0.162

Table 2. Results of Marketed Formulation

Sl No	Label Claim (mg/Tablet)		Amount Obtained (mg/Tablet)		Percentage Label Claim %	
	LOPI	RITO	LOPI	RITO	LOPI	RITO
1	200	50	201	51.6	101	103.2
2	200	50	204	51.6	102	103.2
3	200	50	206	51.6	103	103.2
4	200	50	204	51.4	102	102.8
5	200	50	204	51.4	102	102.8
6	200	50	205	51.2	102.5	102.4

Table 3. Statistical Evaluation of Marketed Formulation

Components	Mean* Percentage Label Claim %	Standard Deviation (SD)	RSD%
LOPINAVIR	102.08	0.6645	0.6510
RITONAVIR	102.93	0.3262	0.3172

*Average of 6 determination

Table 4. Results of Recovery Studies

Level of % Recovery	Amount of Drug Present (µg/mL)		Amount of Standard Added(µg/mL)		Drug Recovery (µg/mL)		Drug Recovery Percentage (%)	
	LOPI	**RITO**	**LOPI**	**RITO**	**LOPI**	**RITO**	**LOPI**	**RITO**
80	100	25	80	20	81.04	21.04	101.3	105.2
	100	25	80	20	80.05	21.02	100.06	105.1
	100	25	80	20	81.04	21.04	101.3	105.2
100	100	25	100	25	100.89	26.26	100.89	105.04
	100	25	100	25	101.6	26.39	100.6	105.56
	100	25	100	25	100.89	26.26	100.89	105.04
120	100	25	120	30	121.54	31.17	101.28	103.9
	100	25	120	30	119.29	31.24	99.40	104.13
	100	25	120	30	120.55	31.15	100.45	103.83

Table 5. Statistical Evaluation of Recovery Studies

Level of % Recovery	Mean* % Recovery		Standard Deviation (SD)		RSD %		Standard Error (SE)	
	LOPI	RITO	LOPI	RITO	LOPI	RITO	LOPI	RITO
80	100.88	105.16	0.7159	0.0577	0.7109	0.0548	0.4133	0.0333
100	100.79	105.21	0.1674	0.3002	0.1660	0.2853	0.0966	0.1733
120	100.37	103.95	0.9421	0.1569	0.9386	0.1509	0.5439	0.0905

*Average of 3 determination

Table 6. Result of Repeatability studies

Sl No	Amount Present (mg/Tablet)		Amount Obtained (mg/Tablet)		Percentage Label Claim %	
	LOPI	RITO	LOPI	RITO	LOPI	RITO
1	200	50	202	51.6	101	103.2
2	200	50	204	51.6	102	103.2
3	200	50	206	51.6	103	103.2
4	200	50	204	51.4	102	102.8
5	200	50	204	51.4	102	102.8
6	200	50	205	51.2	102.5	102.4

Table 7. Statistical Evaluation of Repeatability

Components	Mean* %Label Claim	Standard Deviation (SD)	RSD%	Standard Error (SE)
LOPINAVIR	102.08	0.6645	0.6509	0.2712
RITONAVIR	102.93	0.3265	0.3172	0.1332

*Average of 6 determination

Table 8. Statistical Evaluation of Inter day Intermediate Precision

Components	Mean* %Label Claim	Standard Deviation (SD)	RSD%	Standard Error (SE)
LOPINAVIR	102.38	0.7830	0.7647	0.1845
RITONAVIR	102.91	0.4765	0.4630	0.1091

*Average of 18 determination

Table 9. Linear Regression Data from Calibration Curves

Method parameter	Lopinavir		Ritonavir	
	240nm	260nm	240nm	260nm
Linearity (µg/mL)	100-500	100-500	10-50	10-50
Slope	0.0008	0.0010	0.0117	0.0027
Intercept	0.0108	0.0008	0.00	0.0059
R^2	0.9997	0.9999	0.9926	0.9887

Table 10. LOD and LOQ

	Lopinavir (µg/mL)		Ritonavir (µg/mL)	
Parameters	**240nm**	**260nm**	**240nm**	**260nm**
LOD	24.43	7.16	2.44	6.43
LOQ	74.03	21.71	7.40	19.5

CONCLUSION

A simple, accurate and cost-effective spectroscopic method has been developed for the estimation of lopinavir and ritonavir combined in tablet dosage form. The method was developed by using IPA-Water mixture in 50:50 ratio. Since the drug is insoluble in water. There were several methods for the estimation of this drug combination. The existing methods mainly uses organic solvents such as methanol, ethanol, acetonitrile etc. Organic solvents are expensive and toxic in nature. In this developed method, Isopropyl

alcohol-water mixture is used as UV solvent, which is least expensive and toxic. This solvent is used as disinfectant. The amount of drug present in the tablet lies between the IP limit. The developed method was validated as per ICH guidelines.

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