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RESEARCH ARTICLE
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STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF LOPINAVIR AND RITONAVIR IN FIXED-DOSE COMBINATION TABLETS.

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Abstract

A stability indicating simple, precise,accurate,rapid and validated HPTLC method for simultaneous estimation of Lopinavir and Ritanavir was successfully developed. The method is based on HPTLC separation of the two drugs followed by densitometric measurements of their spots at 266 nm.This method is based on HPTLC separation followed by UV detection at 266 nm. The separation was carried out on merck TLC aluminium sheets precoated with silica gel 60 F_{254} using a camag Linomat5 The mobile consists of Benzene: ethanol : aceticacid (7:3: 0.4% v/v) . Calibration curves were linear in range of 800-4800 ng/band and 200- 1200 ng/ band Lopinavir and Ritonavir respectively . LPV and RTV gave sharp and well defined peaks at Rf value are 0.63 and 0.44 respectively, Stress degradation study shows that sample degraded with acid and base hydrolysis, under oxidation, thermal and photolytic stress conditions.. No chromatographic interferences from the tablet excipients were found. The method was validated in accordance with the requirements of ICH guidelines.

Keywords: Lopinavir(LPV) and Ritonavir(RTV), HPTLC, Forced degradation, Validation.

Conflict of interest: None

1. Introduction.

Lopinavir[1-3] (Figure1)is a dicarboxylic acid diamide that is amphetamine is substituted on nitrogen by a (2,6-dimethylphenoxy)acetyl group and on the carbon alpha- to nitrogen by a (1S,3S)-1-hydroxy-3-{[(2S)-3-methyl-2-(2-oxotetrahydropyrimidin-1-yl)butanoyl]amino}-4-phenylbutyl group· Ritonavir[4-6] is an L-valine derivative that is L-valinamide in which alpha-amino group has been acylated by a [(2-isopropyl-1,3-thiazol-4-yl)methyl]methylcarbamoyl group and in which a hydrogen of the carboxamide amino group has been replaced by a (2R,4S,5S)-4-hydroxy-1,6-diphenyl-5-{[(1,3-thiazol-5-ylmethoxy)carbonyl]amino}hexan-2-yl group.

Ritonavir and Lopinavir are antiretroviral of protease inhibitor class drugs . It is used against HIV infections as a fixed-dose combination with another protease inhibitor, ritonavir (lopinavir/ritonavir). Lopinavir(Figure2) was previously under investigation in combination with ritonavir for the treatment of COVID-19 caused by SARS-CoV-2·

The chemical structures of Lopinavir and Ritonavir shown in the figures 1 &2

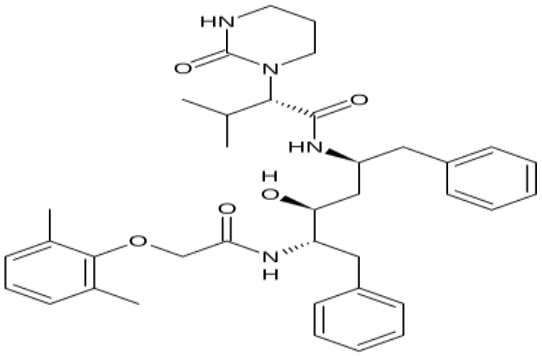


Fig 1: Chemical structure of Ritonavir

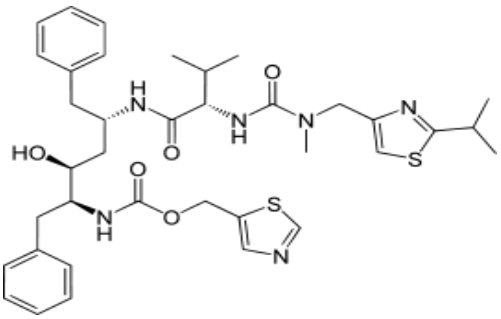


Fig 2: Chemical strure of Lopinavir

Literature survey reveals that very few analytical methods have been reported for estimation of Ritonavir and Lopinavir [7-12].. Hence, considering inherent advantage of HPTLC over HPLC,

The aim and objective of the present work was to develop and validate a stability indicating simple, precise, sensitive high performance thin layer chromatography method for estimation of Ritanavir and Lopinavir in its bulk and tablet dosage form and validate as per International Conference on Harmonization (ICH) Q2 (R2) guidelines.[13-19]

2.Experimental:

2.1.Materials and Reagents

Lopinavir and Ritonavir were kindly supplied as a gift sample by Spectrum Research Labs ,Hyderabad.India. All chemicals and reagents employed were of analytical grade and were purchased from Rankem, Avantor performance material India limited Mumbai. and used without further purification.

2.2. Instrumentation and chromatographic condition.

The HPTLC system (CAMAG, Switzerland) consisted of Linomat V auto sampler connected to a nitrogen cylinder, a twin trough chamber (20 × 10cm), a derivatisation chamber, a plate heater, TLC Scanner IV (Camag Muttenz, Switzerland), UV lamps and UV cabinet with dual wave length and win CATS software were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose.(Figure.3)

Fig.3 Instrumentation of HPTLC

The HPTLC analysis was performed on Pre-coated SilicaGel 60 F_{254} HPTLC plates (20 × 10 cm, layer thickness 0.2 mm (E.Merck KGaA, Darmstadt, Germany). HPTLC plates were pre-washed with 10 mL of methanol and activated at 80ºC for 5 min before application of sample. The standard and formulation samples of Lopinavir& Ritanavir were spotted using a Linomat 5 auto sampler fitted with a 100 μL Hamilton syringe (CAMAG, Muttenz, Switzerland) and operated with settings of a band length of 3.5 mm; band distance of 7.2 mm; distance from the side of plate of 10mm; and distance from the bottom of the plate of 10 mm. The plates were developed to a distance of 70mm in a mobile phase consisting of Benzene: methanol: acetic acid (7: 3: 0.4, *v/v/v*) and development was carried out in twin trough chamber (20 x 10 cm) presaturated with the mobile phase. The developed HPTLC plates were air dried and densitometric scanning was performed onCAMAG TLC scanner III in absorbance mode equipped with WINCATS planar chromatography manager (version 1.4.6) software. The spots were analyzed at a wavelength of 266nm. The scanning of the spots was done at a rate of 20mm/s. Evaluationwas performed using linear regression analysis via peak areas.

Stationary phase: Precoated silica gel 60 F_{254} HPTLC aluminium plates (20 × 10 cm, 0.2mm thick).

Mobile phase: Benzene: methanol: acetic acid (7: 3: 0.4, *v/v/v*)

Saturation time: 20 minutes.

Wavelength: 266 nm.

Lamp: Deuterium

2.3.Preparation of Standard Solutions of LPV and RTV.
An accurately weighed 40 mg of LPV and 10 mg of RTV were transferred to 50 ml volumetric flask and volume was adjusted to mark to obtain concentration 800 µg /ml of LPV and 200 µg/ ml of RTV

3.Application of the Proposed Method for Simultaneous Estimation of the Drugs in Laboratory Mixture.
Accurately weighed 40 mg of LPV and 10 mg of RTV were transferred to 50 ml volumetric flask, dissolved in methanol and volume was adjusted to mark. Appropriate volume 3 µl containing 2400 ng of LPV and 600 ng of RTV was spotted on TLC plate. The plate was developed, dried and scanned as described The concentration was determined by regression equation(Table 1)

4.Application of the Proposed Method for Simultaneous Estimation of the Drugs in Tablets
To determine the content of LPV and RTV simultaneously in conventional tablets (label claim 200 mg LPV and 50 mg RTV); twenty tablets were accurately weighed, average weight determined and ground to a fine powder. A quantity of powder equivalent to 40 mg was transferred into 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to mark with same solvent. The resulting solution wasfiltered using 0.45 µm filter (Millifilter, MA). 0.4µL of the above solution applied on TLCplate followed by development and scanning as described in section 2.2.The analysis was repeated for six times. LPV and RTV gave sharp and well defined peaks at Rf 0.63 and 0.44, respectively, when scanned at 240 nm. The concentration of drugs was determined from linear regression equations and % label claim was calculated, (Table 2).

5.Analytical Method Validation.
The developed method was validated for different parameters like linearity, precision, accuracy, specificity, ruggedness, robustness, LOD and LOQ as per ICH Q2(R_1) guidelines .

5.1. Accuracy
Recovery study was carried out by over spotting 80%, 100% and 120% of the standarddrug solution

of LPV and RTV and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check the recovery of the drug at different levels in formulation. The results of % recovery was calculated (Table .3).

5.2.Precision (Intra- day and Inter- day precision).

Precision of the method was studied as intra-day and inter-day variations. Intra-day variation was determined by analysing three different concentrations 1600, 2400 and 3200 ng/spot of LPV and of RTV 400, 600 and 800 ng/spot, for three times within a day.

Inter-day precision was assessed using same concentration of drug (mentioned above) and analysed it for three different days, over a period of week (Table 4.)

5.3.Linearity Studies of LPV and RTV

Aliquots portion in the range 1 - 6 µl was applied on TLC plate to obtain concentration 800,1600,2400,3200,4000 and 4800 ng/spot of LPV and 200,400,600,800,1000 and 1200 ng/spot of RTV. The plates were developed, dried and scanned as described above.

Thecalibration curves were constructed by plotting peak areas against the corresponding concentrations of both the drugs (ng/spot), individually. The calibration curves (Figure 4 & 5) linearity data are reprented(Table 5&6).

5.4.Repeatability

Repeatability of sample application was assessed by spotting 2400 ng/spot for LPV and 600 ng/spot for RTV of drug solution six times on a TLC, followed by development of plate. The results are reprented (Table. 7)

5.5.Specificity

The mobile phase designed for the method resolved both the drugs very efficiently; The R_f value of **LPV** and **RTV** was found to be **0.63 ± 0.03** and **0.48 ± 0.03,** respectively.(Figure.6) A typical absorption spectrum of **LPV** and **RTV** is shown (Figure 7**).** The peak purity of RTV-extracted from capsule and standard RTV was assessed by correlating the spectra of RTV at the peak-start (S), peak-apex (A) and at the peak-end (E) positions. Correlation between these spectra indicated the purity of RTV peak {correlation *r*(S, M) = 0.998, *r* (M, E) =0.999}(Figure8) The peak purity of LPV was assessed by correlating the spectra of standard LPV and LPV- extracted from capsule at the peak-start (S), peak- apex (A) and at the peak-end (E) positions. Correlation between these spectra indicated purity of LPV peak {correlation *r*(S, M) = 0.998, *r* (M, E) = 0.999}; (Figure9)Thus, it can be concluded that no impurities or degradation products were found with the peaks of standard drug solutions.

5. 6.Sensitivity

The sensitivity of measurement of LPV and RTV by the use of the proposed method was determined in terms of the LOD and LOQ. The LOD and LOQ were calculated using equation LOD = 3.3 X N/B and LOQ = 10 X N/B; Where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. Mixed stock solution of LPV and RTV was prepared and different volume in the range 800 - 1000 ng/band of LPV and 200 - 400 ng/band of RTV were applied in triplicate. The LOD and LOQ for LPV were found to be **24.84 ng** and **75.30ng**, respectively. For RTV the LOD and LOQ was found to be **15.83 ng** and **54.97 ng**, respectively.

5 7. Ruggedness and Robustness

Ruggedness of the proposed method was studied by two different analysts using the same experimental and environmental conditions. The spots 2400 ng of LPV and 600 ng of RTV were

applied on TLC plates. The development and scanning of spots were performed as discussed in section 6. This procedure was repeated in triplicates Robustness was studied in six replicate at the concentration level of 2400 ng/spot for LPV and 600 ng/spot for RTV. In this study, seven parameters (mobile phase composition, mobile phase volume, development distance, relative humidity, duration of saturation, timefrom spotting to chromatography and chromatography to spotting) were studied and the effects on the results were examined; the results are represented (Table 8&9)

5.8.Detection limit and Quantification Limit

A limit of detection (LOD) and a limit of quantification (LOQ) were calculated according to the formula:

LOD = 3.3 σ/s

LOQ = 10 σ/s

Where, 'σ' is the standard deviation of 'y' intercept of regression line and 's' is the slope of the calibration curve.

The LOD and LOQ for LPV were found to be **24.84 ng** and **75.30ng**, respectively. For RTV the LOD and LOQ was found to be **15.83 ng** and **54.97 ng**, respectively.

5. 9.Preparation of sample solution for Force degradation studies

To assess the stability indicating property of the developed HPTLC method, stress studies were carried out under ICH recommended conditions. Forced degradation of Lopinavir & Ritanavir was carried by exposing the bulk sample to acidic, alkaline, oxidative, photolytic, dry heat and neutral conditions. Theaim was to study the ability of the proposed method to measure the analyte response in presence of its degradation products (Table10)

a)Acid and base induced degradation

The 10 mg of LPV and RTV were separately dissolved in 10 ml methanolic solution of 1M HCl and 1 M NaOH. These solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 1 ml of above solutions were taken, neutralized and diluted up to 10 ml with methanol. The resultant solution were applied on TLC plates in triplicates (10 µl each, i.e. 1000 ng/spot). The chromatograms were run as described (Figure10a &11a)

b) Hydrogen peroxide – induced degradation

The 10 mg of LPV and RTV were separately dissolved in 10 ml of methanolic solution of hydrogen peroxide (10%, v/v). The solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 1 ml of above solutionwere taken and diluted up to 10 ml with methanol. The resultant solution were applied on TLC plate in triplicate (10 µl each, i.e. 1000 ng/ spot). The chromatograms were run as described (Figure. 10.b& 11.b)

c) Dry heat degradation products

LPV 10 mg and RTV 10 mg were stored at 55^0 C for 3 h in oven separately. They were transferred to 10 ml volumetric flask containing methanol and volume was made up to the mark. 1.0 µl (1000 ng / spot) was applied on TLC plate in triplicate and chromatogram were run as described (Figure 10.c& 11.c)

d) Light heat degradation products

The 10 mg of LPV and RTV were dissolved in 10 ml of methanol separately. The solutionswere kept in the sun light for 8 h. The 1 ml of above solutions were taken and diluted up to 10 ml with methanol. The resultant solutions were applied on TLC plate in triplicate (10 µleach, i.e. 1000 ng / spot). The chromatograms were run as described in section 6. The chromatogram of samples degraded with acid, base, hydrogen peroxide and light showed well separated spots of pure LPV and RTV as well as some additional peaks at different R_f

are shown . (Figure 10.d & 11.d)

6.Statistical Analysis

The proposed method was compared with the reported method for determination of drug indosage form by Analysis of Variance (ANOVA) test (Table 11.)

The results obtained in assay were analyzed by ANOVA to assess that no significant difference between the reported and proposed method F stat< F crit (P>0.05) indicates method is not significant, (Figure12.a, 12.b &12.c**)**

Table 1: Analysis of bulk material

Component	Amount Taken (ng/band)	Amount Found (ng) □ SD	%RSD [n=6]
LPV	2400	2380.84□ 25.18	1.05
RTV	600	596.75□ 5.35	0.89

Table 2: Analysis of marketed Formulation

Brand Name: LOPIMUNE TABLETMfg. By: CIPLA.

Batch No.: BF90004Average weight: 0.6248 gm

Component	Label Claim [mg]	Amount Found ± SD [ng]*	% Claim	Label % RSD[n=6]
LPV	200	2380.84□ 25.18	99.20	1.05
RTV	50	593.36 ±1.25	98.89	1.25

***** Mean of six estimations

Table 3: Recovery studies for accuracy.

Components	Initial Amount [ng/band]	Amount added (%)	Amount recovered ± S.D.[ng/band][n=3]	% Recovered	% RSD
	1600	80	1273	99.52	1.05
LPV	1600	100	1606	100.41	1.28
	1600	120	1906	99.30	0.75
	400	80	322.81	100.87	1.38
RTV	400	100	399.34	99.83	1.45
	400	120	477.89	99.56	0.64

Table 4: Precision Studies (Intra- day and Inter- day)

Drugs	Conc.[ng/band]	Intra day Amount found [ng] Mean ± SD [n= 3]	% RSD	Inter day Amount found [ng] Mean ± SD [n= 3]	% RSD
	1600	1588.52 ± 3.21	0.202	1593.22 ±6.71	0.421
LPV	2400	2411 ±5.65	0.234	2402.58 ±11.38	0.474
	3200	3189 ±12.72	0.399	3197.28 ±8.08	0.250
	400	395.50 ±4.98	1.261	403.05±4.89	1.214
RTV	600	591.90±4.82	0.814	606.81±5.15	0.849
	800	792.86±9.45	1.192	809.81±6.66	0.822

Table 5: Linearity Study of LPV

Concentration (ng/band)	Area ± SD	%RSD (n=6)
800	2887 ± 34.95	1.21
1600	4672 ± 55.32	1.18
2400	6472 ± 51.33	0.79
3200	8173 ± 88.33	1.08
4000	9519 ± 134.19	1.40
4800	11136 ± 173.68	1.55

Table 6: Linearity Study of RTV

Concentration (ng/band)	Area ± SD	%RSD (n = 6)
200	1834 ± 18.72	1.02
400	2935 ± 24.08	0.82
600	4140 ± 37.88	0.91
800	5159 ± 44.48	0.86
1000	6154 ± 46.26	0.75
1200	7054 ± 58.29	0.82

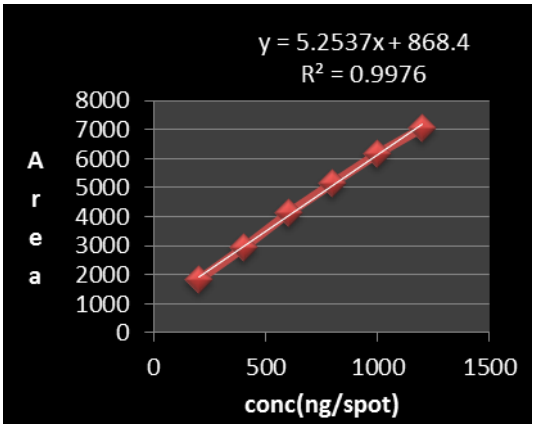


Fig 4.: Calibration curve of LPV

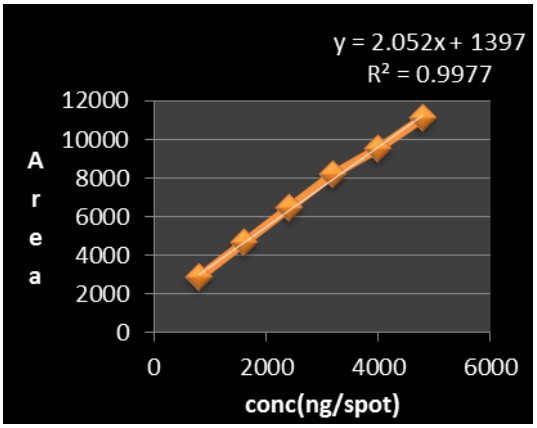


Fig 5.: Calibration curve of RTVY = 2.052 X + 1397; where, Y = 5.2537 X + 868.4; where,

Correlation coefficient = 0.9977; Correlation coefficient = 0.9976;Slope = 2.052; Intercept = 1397Slope = 5.2537; Intercept = 868.4

Table 7: Results of Repeatability

Component	LPV 2400 [ng/band]	RTV 600 [ng/band]
Peak Area	3925	6295
	3978	6248
	3995	6214
	4085	6317
	4012	6421
	4069	6196
Mean	4010.66	6281.83
S.D.	59.29	82.32
%R.S.D.	1.47	1.31

Table 8: Results of Ruggedness Studies

Analyst	% Amount foundLPV[*n* = 3]	% RSD	% Amount found RTV[*n* = 3]	% RSD
I	99.42	1.50	99.26	1.73
II	99.62	0.90	99.48	1.40

Table 9: Results of Robustness Studies

Parameters	**LPV**		**RTV**	
Mobile phase composition	**SD of peak area[n = 6]**	**% RSD**	**SD of peak area[n = 6]**	**% RSD**
Benzene : methanol: acetic acid (7: 3: 0.4, *v/v/v*)	39.25	1.65	9.31	1.56
Benzene : methanol: acetic acid (9: 3: 0.4, *v/v/v*)	46.74	1.24	34.45	1.17
Mobile phase volume				
10.4 ml	14.37	0.76	45.15	0.45
12.4 ml	24.42	0.24	37.03	1.05
Development distance				
7 cm	13.67	0.65	16.23	0.65
7.5 cm	16.67	0.45	21.13	0.56
8 cm	35.33	1.17	21.56	0.65
Relative humidity				
55	12.23	0.28	33.32	0.45
65	23.34	1.21	34.06	0.78
Duration of saturation				
20 min	23.12	0.72	21.15	0.65
25 min	26.34	0.89	28.24	1.09
30 min	29.67	1.12	37.56	1.45
Activation of prewashed TLC plates				
8 min	21.01	0.78	18.28	0.97
10 min	27.64	0.88	28.04	0.46
12 min	36.04	1.33	39.72	1.11
Time from spotting tochromatography	24.41	0.86	19.32	1.45
Time from chromatography to scanning	31.09	0.98	34.66	0.87

Table 10: Forced degradation of LPV and RTV

Sample exposure condition	**Number of degradation products (R_f values)**		**Drug remained (1000 ng/spot)**		**Recovery (%)**	
	LPV	**RTV**	**LPV**	**RTV**	**LPV**	**RTV**
1 M HCl, 8h,RT	2 (0.48,0.51)	2 (0.25,0.38)	956.52	946.89	95.65	94.68
1M NaOH,8h, RT	3 (0.29, 0.56)	2 (0.28,0.31)	945.23	945.23	94.52	94.52
10%H_2O_2,8h,RT	1(0.58)	1 (0.25)	959.12	975.65	95.91	97.56
Photo, 8 h	No degradation	No degradation	999.12	997.42	99.91	99.74
Heat, 3H, 55^0C	No degradation	No degradation	996.23	991.28	99.62	99.12

[a]RT = Room Temperature

Table .11: Comparison of methods for the determination of LPV and RTV intablets by ANOVA test

Sample	Reported method		Developed method	
	LPV	RTV	LPV	RTV
1	98.12	99.67	99.27	98.98
2	99.07	99.45	99.02	98.67
3	98.03	99.89	99.21	98.65
4	98.43	98.65	99.24	98.64
5	99.56	98.67	99.36	99.15
6	98.37	98.89	99.12	99.25

ANNOVA: Two factor with replication

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.129067	1	0.129067	0.704666	0.411142	4.351243
Columns	0.129067	1	0.129067	0.704666	0.411142	4.351243
Interaction	1.2696	1	1.2696	6.931644	0.015952	4.351243
Within	3.6632	20	0.18316			

Fstat <F crit (P>0.05)

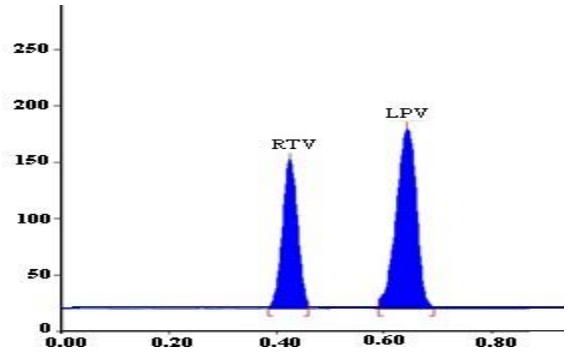


Fig 6.c:Typical HPTLC Chromatogram of LPVRTV (R_f = 0.44) and LPV (R_f = 0.63) standard solution

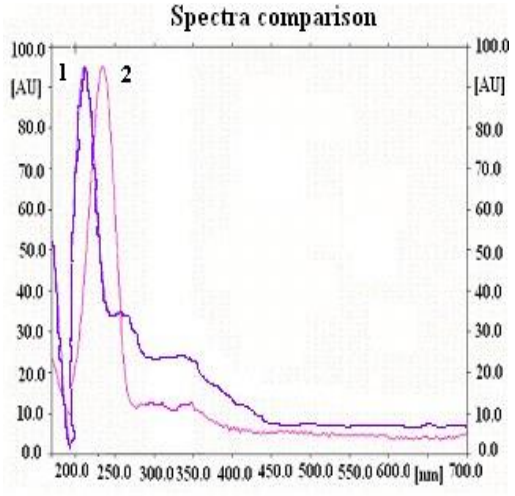


Fig 7.d:Typical overlain spectra of standard (1) and RTV (2) standard solution

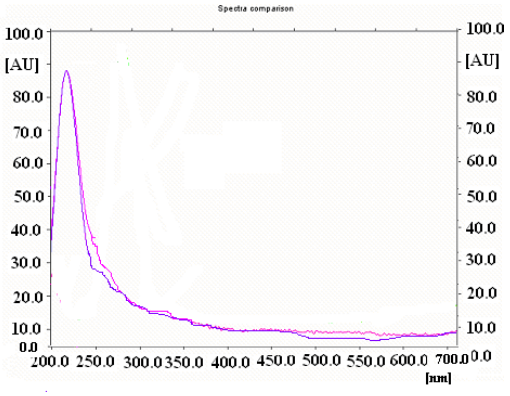


Fig 8: Peak purity spectra of RTV

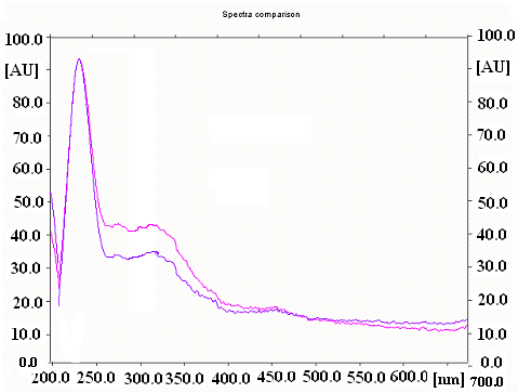
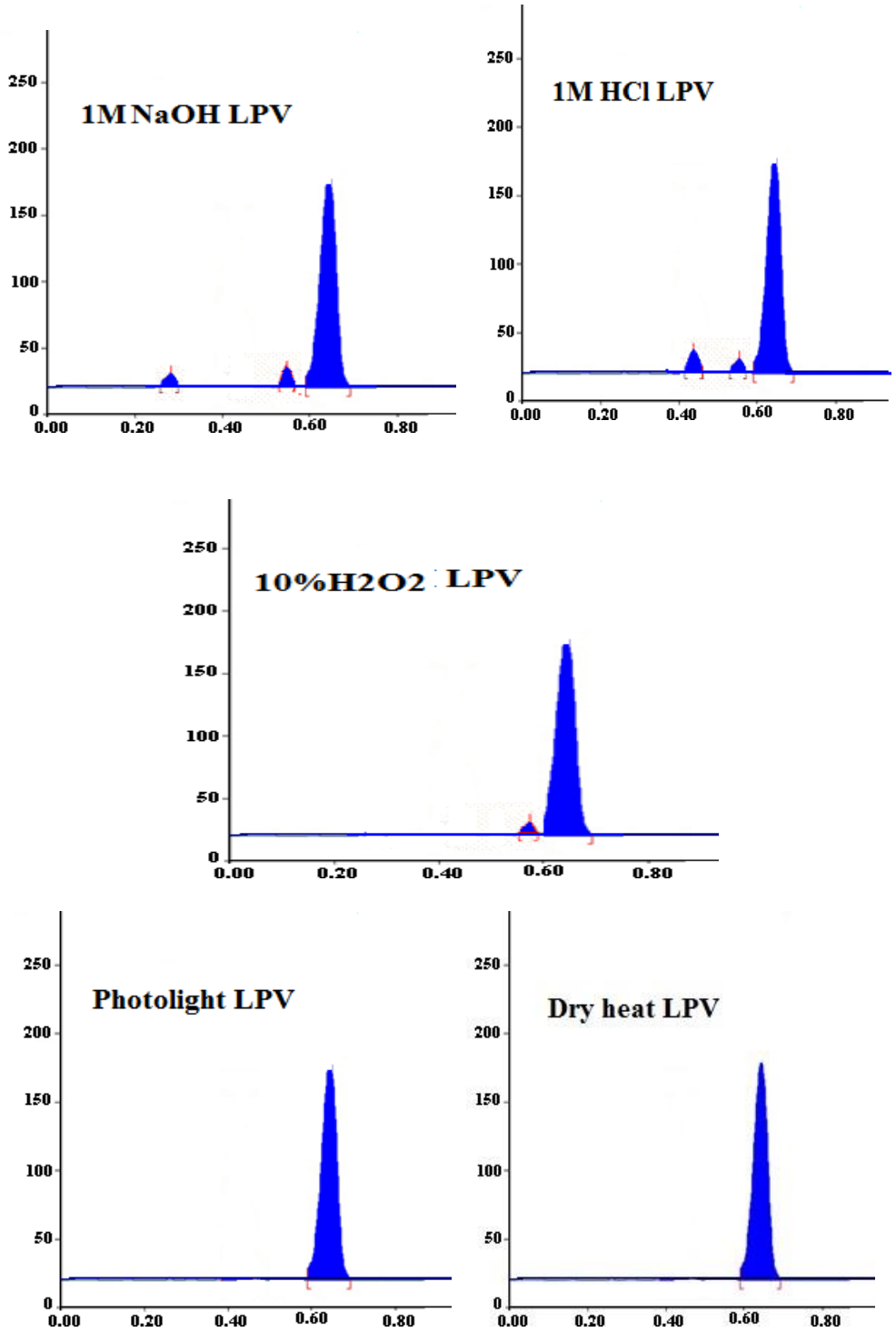
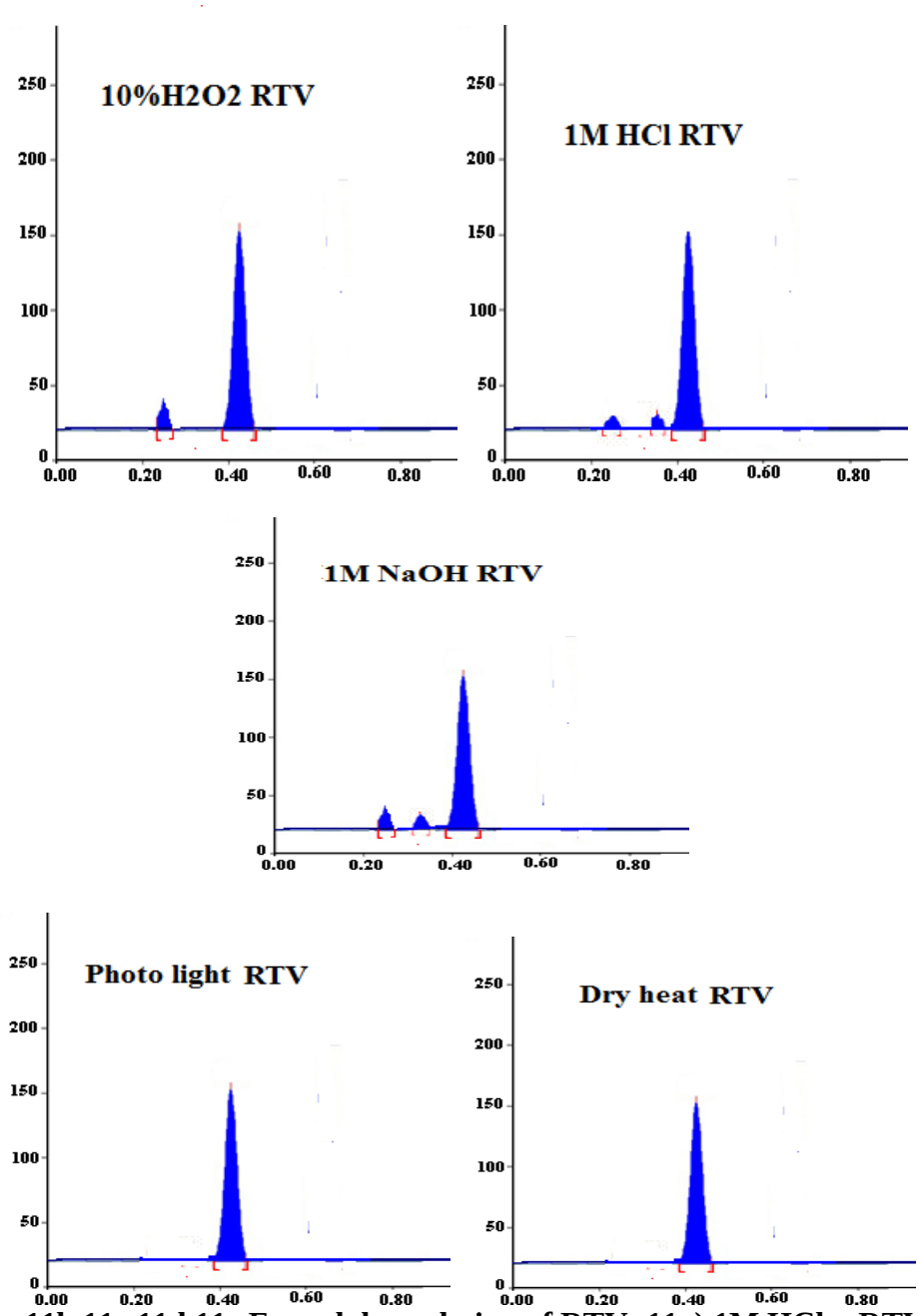


Fig 9 : Peak purity spectra of LPV

Extracted from RTV – LPV Tablet, Extracted from LPV – RTV Tablet and standard RTV scanned atand standard LPV scanned at the peak-start, peak-apex andpeak-start, peak-apex and peak-end positions of the spotpeak-end positions of the spot (Correlation > 0.99) (Correlation > 0.99)

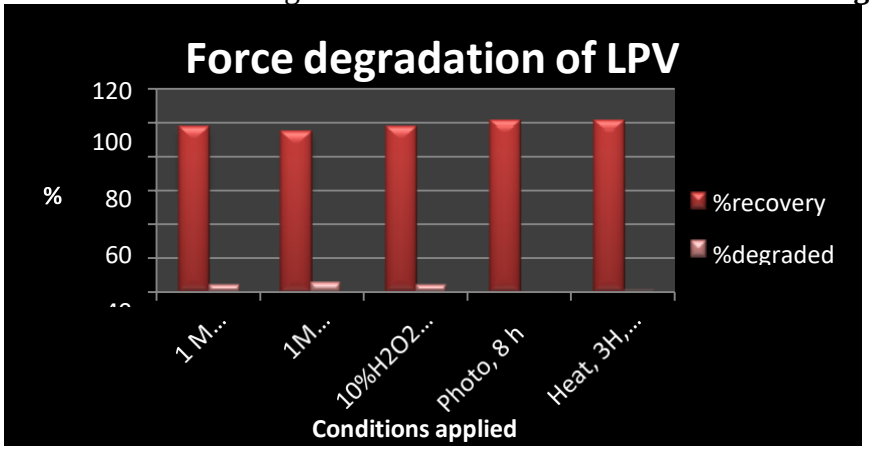


Figures Forced degradation of LPV :10.a) 1M HCl + LPV ;10.b) 1M NaOH + LPV 10.c) **10% H_2O_2 + LPV ; 10.d) Dry heat LPV ; 10.e) Light heat LPV**



Figures 11a,11b,11c,11d,11e Forced degradation of RTV :11a) 1M HCl + RTV ;11b) 1M NaOH + RTV ; 11c)10 % H_2O_2 + RTV ; 11 d) Dry heat RTV; 11e) Light heat RTV

Graphical representation for force degradation for LPV and RTV are shown in **Fig.12 a.&.12.b.**



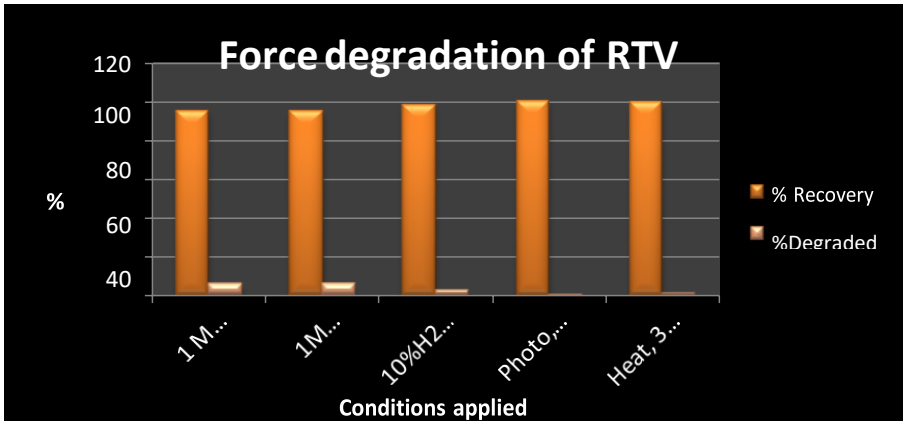


Fig. 12 a,12b Forced degradation of LPV and RTV

It was observed that the developed analytical method is stability-indicating one.

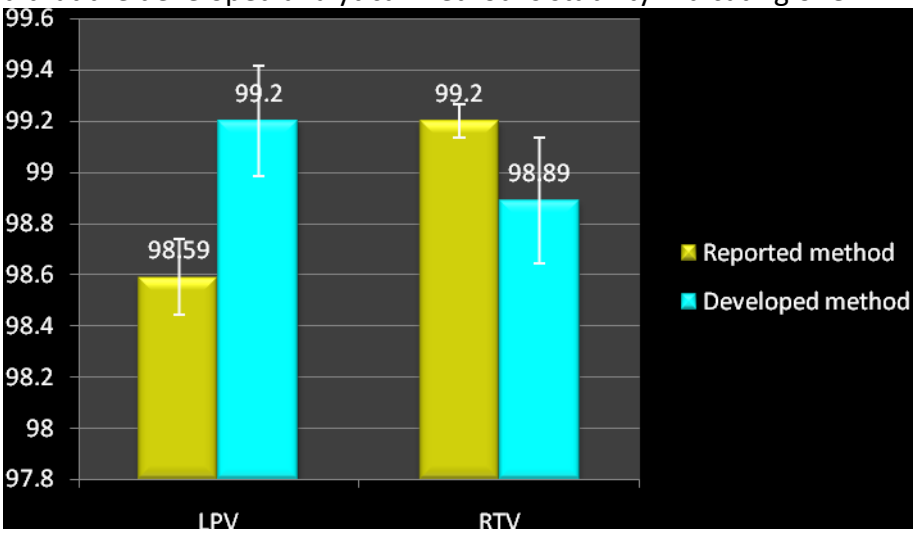


Figure 12c Comparison between reported and developed HPTLC method.

7.Discussion

Stability indicating HPTLC method for the simultaneous analysis of Lopinavir and Ritonavirin fixed-dose Combination tablets has been developed.The method is based HPTLC separation of the two drugs followed by densitometric measurements of their spots at **266nm**. The separation was carried out on Merck TLC aluminium sheets of silica gel 60F 254using **Benzene: methanol: acetic acid (7: 3.:0.4, v/v/v)** as a mobile phase. LPV and RTV gavesharp and well defined peak at **R_f 0.63 and 0.44**, respectively. Calibration curves were foundto be linear in range **800-4800 ng/spot** and **200-1200 ng/spot** for LPV and RTV, respectively.The linear equations for the calibration plots were **Y = 2.052x +1397and Y = 5.253x +868.4,**with correlation coefficient (r^2) being0.997 and0.997 for LPV and RTV,respectively. The proposed method was applied for pharmaceutical formulation and % labelclaim of LPV and RTV were found to be **99.20 % and 98.89 %** respectively. The recoverystudies were carried out at 80, 100, 120 % level and % recovery of LPV was found to be **99.30 – 100.41 %** and for **RTV 99.56-100.87 %.**

The % RSD values less than 2 indicative of accuracy of the methods. Precision of the method was determined in the terms of intra-dayand inter-day variation and repeatability. Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. ForLPV the % RSD was found to be **0.250-0.474** and for RTV % RSD was found to be **0.822-1.214**. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week. For LPV the % RSD was found to be **0.202-0.399** and for RTV % RSD was found to be **0.814-1.261**. Repeatability of sample

application was assessed by spotting (2400 ng/spot of LPV and 600 ng/spot of RTV) of drug solution seven times on a TLC, followed by development of plate and recording the peak area for seven spots. The % R.S.D. for peak area values of LPV and RTV was found to be **1.47 and 1.31,** respectively. The standard deviation of peak areas was calculated for each parameter and %R.S.D. was found to be less than 2%. The low %R.S.D. values indicated robustness of the method. The ruggedness of the proposed method was evaluated by two different analysts. The results for LPV and RTV were found to be **99.42 %, 99.62 % and 99.26 %, 99.48 %,** respectively.

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9.Conclusion.

It is found that the developed HPTLC technique is quite simple, authentic, definite, reproducible, sensitive, favorable, specific and economical. It can become efficient analytical tool for routine quality control of Lopinavir & Ritanavir in bulk drug and its pharmaceutical dosage forms.

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