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Research Article

Quantification and stability aspects of Luliconazole in bulk and pharmaceutical dosage forms by UV spectroscopy

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ABSTRACT

Two simple and economical UV spectroscopic methods were developed for the estimation of Luliconazole in creams. The drug showed maximum absorption at 294 nm, both in 0.1N HCl and phosphate buffer (pH 2.0) in the fundamental spectra (D^0). The same spectra were derivatized into first derivative (D^1) and the $dA/d\lambda$ was measured at 315 nm in 0.1N HCl and 317 nm in phosphate buffer (pH 2.0). In both the methods the drug obeyed Beer-Lambert's law in the concentration range of 2-30 µg/mL in 0.1N HCl and 10-30 µg/mL in phosphate buffer (pH 2.0). The linear regression equations were calculated to be y = 0.0504x + 0.0102 (R^2 = 0.9991) for D^0 and y = 0.0025x + 0.0002 (R^2 = 0.9991) for D^1 in 0.1N HCl, y = 0.0637x + 0.0181 (R^2 = 0.999) for D^0 and y = 0.0025x + 0.0006 (R^2 = 0.999) for D^1 in phosphate buffer (pH 2.0). An acceptable recovery in the range of 98 ± 0.01 – 102 ± 0.001 % indicates accuracy as well as non-interference from excipients in the present method. The intraday and inter day precision results were within 2 % RSD indicating the preciseness of the methods. The methods were applied for quantification of Luliconazole in marketed creams and the assay was obtained as 98.53 % w/w against the label claim. The methods were also applied to study the stability aspects of the drug in a variety of conditions like acid, base and oxidative stress along with thermal and photolytic stress conditions. The drug showed altered absorbance in basic and photolysis conditions. The methods were validated statistically as per the ICH guidelines.

Keywords: Luliconazole, UV spectroscopy, Stability, Validation, ICH.

INTRODUCTION

Luliconazole (LCZ), chemically named as (2E)-2-[(4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile (Fig.1.) is a broad-spectrum imidazole that is active against various fungi including *Tinea, Candida, Aspergillus, Trichophyton* and *Epidermophyton*. It has a molecular formula $C_{14}H_9Cl_2N_3S_2$, molecular weight of 354.28 and melting point in the range 121-125°C [1]. Luliconazole is used for the treatment of interdigital tinea pedis, tinea cruris, and tinea corporis [2-3]. The mode of action of Luliconazole against dermatophytes is unknown, Luliconazole appears to inhibit ergosterol synthesis by inhibiting the enzyme lanosterol demethylase. Inhibition of this enzyme's activity by azoles results in decreased amounts of ergosterol, a constituent of fungal cell membranes, and a corresponding accumulation of lanosterol [4, 5]. Luliconazole may be metabolized by CYP2D6 and 3A4 [6]. Luliconazole, an imidazole antifungal medication available as a 1% topical cream, is indicated for the treatment of

athlete's foot, jock itch, and ringworm caused by dermatophytes such as *Trichophyton rubrum*, *Microsporum gypseum* and *Epidermophyton floccosum*.
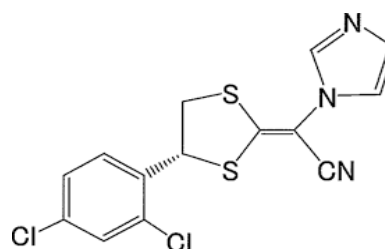
Figure1: Structure of Luliconazole

Review of literature for Luliconazole analysis revealed very few methods such as LC-MS/MS [7] method (toe nails) HPLC for related substances [8], HPTLC method for assay in formulation and biofluid [9], and a single UV spectroscopic method [10] (area under curve) have been reported for assay

of Luliconazole. However, there is no simple method reported for the detection of the drug in pharmaceutical formulation by UV spectrophotometry. So, an attempt has been made to establish a simple, fast, accurate and economic method for determination of Luliconazole in bulk powder and its dosage forms, which can be used in quality control laboratories. This paper reports a study on the development of new validated UV- spectrophotometric methods for the quantitative determination of Luliconazole in creams using 0.1 N HCl and phosphate buffer (pH 2.0).

MATERIALS AND METHODS

A double beam UV-Visible Spectrophotometer (UV-1800 Shimadzu, Japan) with matching quartz cuvettes was used to measure absorbance. All weights were taken on an electronic balance (AUX 220 Shimadzu, Japan).

Reference standard of Luliconazole was obtained as a gift sample from Sun Pharma Ltd. and the pharmaceutical dosage form (cream, Lulifin®) containing 1% w/w of Luliconazole was procured from local pharmacy. Hydrochloric acid, sodium hydroxide, hydrogen peroxide, potassium dihydrogen phosphate (AR grade) were procured from Qualigens and distilled water was used throughout the study.

Preparation of calibration curve: A stock solution was prepared by dissolving 10.0 mg of Luliconazole in 10 mL of methanol in a suitable volumetric flask (1000 µg/mL). Working standard solutions of Luliconazole were prepared by pipetting 1mL of stock solution and diluting to 10 mL with 0.1N HCl and phosphate buffer (pH 2.0) in separate volumetric flasks (100 µg/mL). Further dilutions were made by transferring suitable aliquots (0.2 – 3 mL) into various 10 mL volumetric flasks and made upto volume with the solvents. The resulting solutions were then scanned in the UV range (200-400nm) in 10 mm matched quartz cells in a UV-Visible double beam spectrophotometer. The drug showed maximum absorption at 294 nm in both the solvents. The same spectra were derivatized into first derivative, the derivative absorbance of minima was measured at 315 nm (0.1N HCl) and at 317 nm (phosphate buffer, pH 2.0).

Estimation of Luliconazole in formulation (cream): For the analysis of Luliconazole in creams, 0.5mg of cream base was taken in a 50 mL volumetric flask, methanol was added in increments, the drug was dissolved by constant stirring and after complete dissolving of cream base the remaining volume was made up with methanol. This solution is filtered and from the filtrate suitable aliquots were diluted with 0.1N HCl and phosphate buffer (pH 2.0). The absorbance of each solution was measured against respective blank at 294 nm.

Method Validation

The methods were validated according to International Conference of Harmonization (ICH) guidelines for validation of analytical procedures [11, 12].

Linearity

Standard solutions of Luliconazole were prepared in the range of 2-30 µg/mL (0.1N HCl) and 10- 30 µg/mL (phosphate buffer, pH 2.0). The absorbance of each solution was measured and calibration curves were constructed by plotting absorbance versus concentrations. Linearity was determined from the regression analysis.

Accuracy

It was analysed by percentage recovery of added standard drug from bulk to fixed concentration of sample solutions.

The standard drug was added at 50, 100 and 150% of the sample concentration, each solution was prepared in triplicate in both the solvents and the absorbance measured to find out the percentage recovery.

Precision

Repeatability was calculated by analysing three independent Luliconazole sample solutions (5, 10, 15 µg/mL), in triplicate, in 0.1N HCl and phosphate (pH 2.0). The intermediate precision was evaluated on 2 consecutive days. The precision was expressed as the percentage relative standard deviation (coefficient of variation).

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) are based on the slope of the calibration curves and standard deviation of y-intercepts of regression lines.

Stability

The developed methods were applied for assessing the stability related aspects of the drug and so the drug was exposed to a series of stress like acidic, basic, oxidative, photolytic and thermolytic conditions. Thermal degradation and photo degradation of drug substance were carried out in solid state. Degradations were performed on a 100 µg/mL solution and finally diluted with 0.1N HCl and phosphate buffer (pH 2.0) to obtain a concentration of 10 µg/mL.

Acid hydrolysis in solution state was performed by treating 1 mL of working standard solution of Luliconazole with 1mL of 0.1 N HCl and heated at 60° for 30 minutes. The drug solution was also tested for stability in the acidic medium by treating with 1mL of 0.1N HCl and kept aside for one day without heating. These solutions were made up to volume with 0.1 N HCl and phosphate buffer pH 2.0 for which the absorbance was measured.

Base hydrolysis in solution state was performed by treating 1 mL of working standard solution of Luliconazole with 1mL of 0.1 N NaOH and heated at 60° for 30 minutes. The drug solution was also tested for stability in the basic medium by treating with 1mL of 0.1N NaOH and kept aside for one day without heating. These solutions were made up to volume with 0.1 N HCl and phosphate buffer pH 2.0 for which the absorbance was measured.

For oxidative stress, an aliquot of the working standard solution was treated with 1mL of 3% H_2O_2 and exposed to various conditions like room temperature (for 30 min.), heating at 60° for 30 minutes and kept aside without heating for 1 day. Finally, the volume was made up with 0.1 N HCl and phosphate buffer pH 2.0 and the absorbance was measured.

Photolytic stress was conducted by exposing the drug in solid state and also in solution form to UV radiation for 30 minutes, volume was made up with 0.1 N HCl and phosphate buffer pH 2.0 and absorbance measured.

For thermal stress, solid sample and solution sample of drug substances were entrusted in a controlled-temperature oven at 80°C for 1 Hr, volume made up with 0.1 N HCl and phosphate buffer pH 2.0.

RESULTS AND DISCUSSION

An attempt was made to develop simple and economical methods for the quantification of Luliconazole in creams. Two UV spectroscopic methods (D^0 and D^1) were developed using 0.1N HCl and phosphate buffer (pH 2.0). In the D^0 method, the drug showed maximum absorption at 294 nm and obeyed Beer-Lambert's law in the concentration range

of 2-30 µg/mL for 0.1N HCl and 10-30 µg/mL for phosphate buffer (pH 2.0). The same spectra were derivatized into first derivative, the absorbance of minima was measured at 315 nm for 0.1N HCl, at 317 nm for phosphate buffer (pH 2.0) and the drug showed linearity in the concentration range

from 2-30µg/mL for 0.1N HCl and 10-30µg/mL for phosphate buffer (pH 2.0). The overlain spectra and calibration curves in both the methods are shown in figure 2,3 and 4,5 respectively.
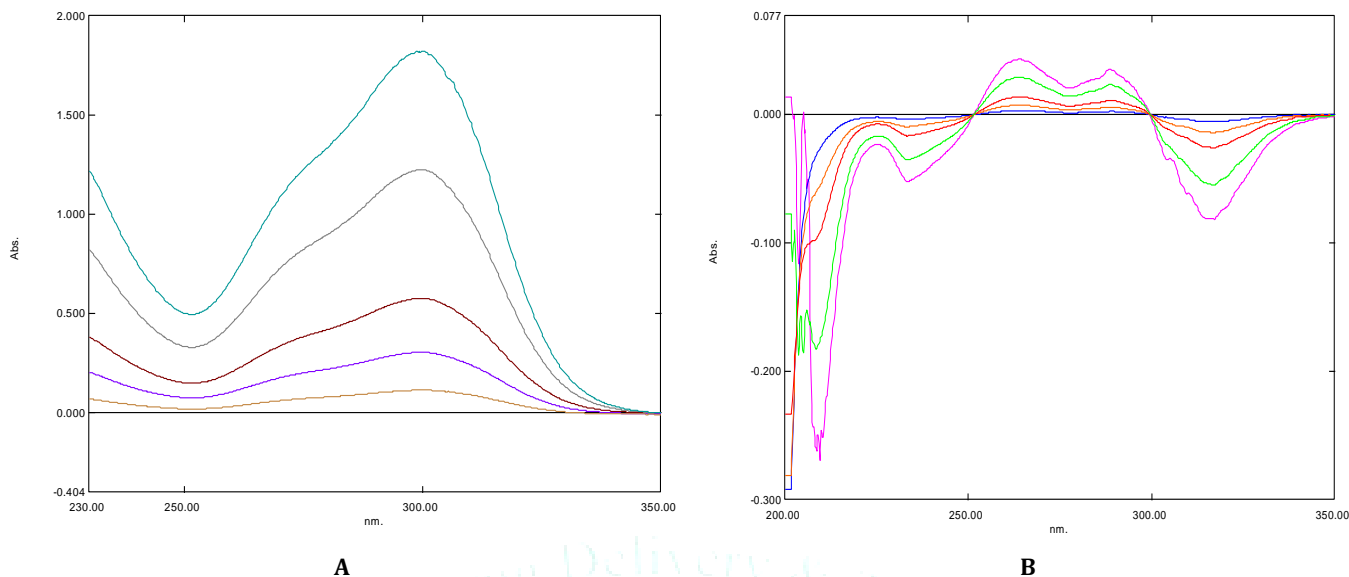

Figure 2: Overlain spectra of Luliconazole in zero order (A) and first order (B) (0.1N HCl)
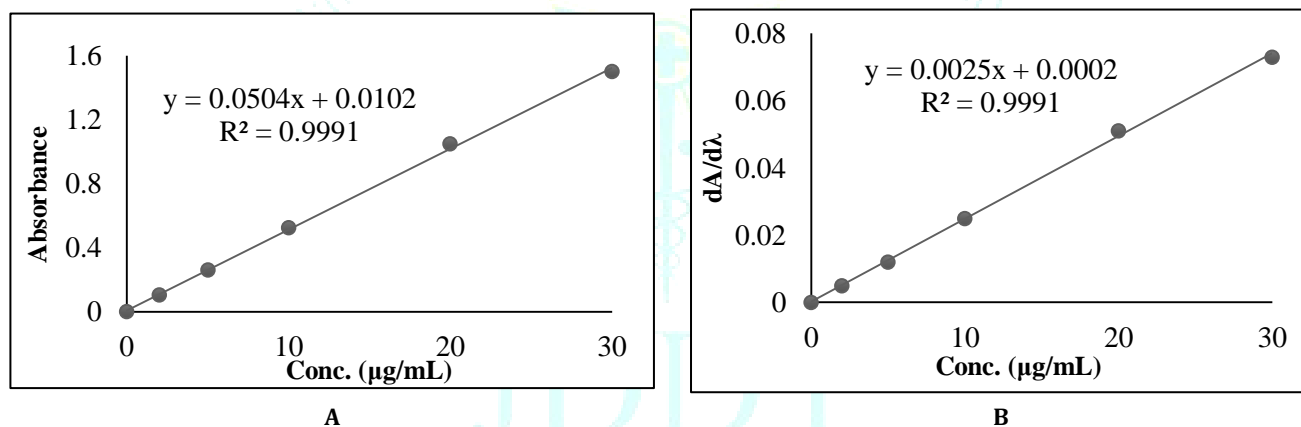

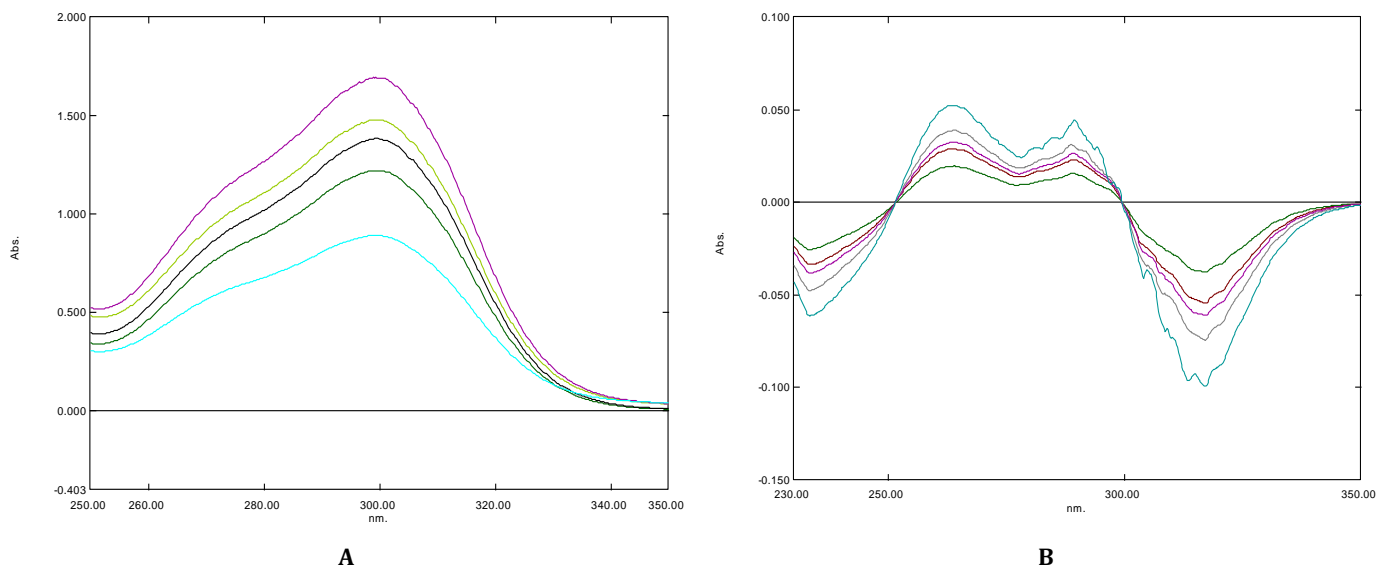**Figure 3:** Calibration curve of Luliconazole in zero order (A) and first order (B) (0.1N HCl)

Figure 4: Overlain spectra of Luliconazole in zero order (A) and first order (B) (Phosphate pH 2.0)
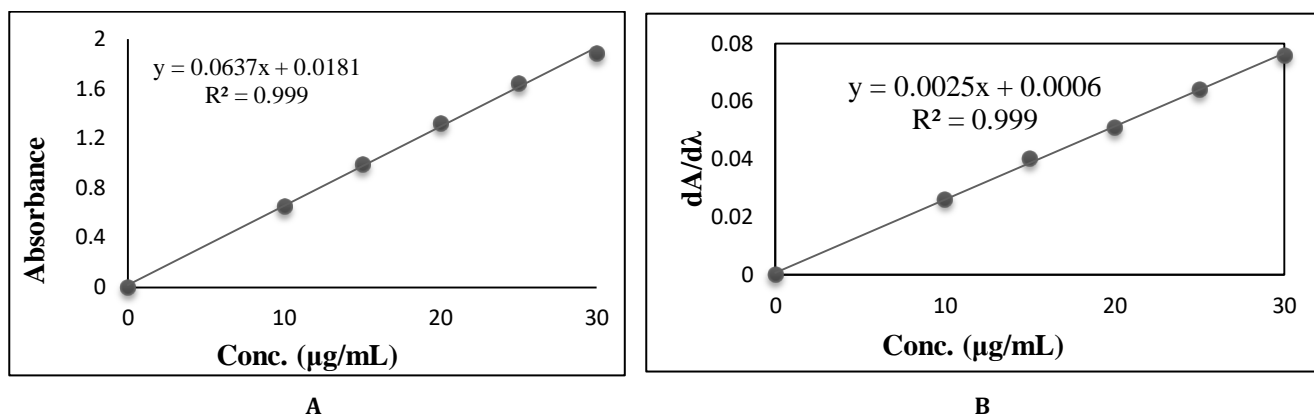
Figure 5: Calibration curve of Luliconazole in zero order (A) and first order (B) (Phosphate pH 2.0)

The methods were validated in terms of accuracy, precision, LOD, LOQ and specificity wherein the results are recorded in table 1 to 6. The accuracy of the method was determined by performing recovery studies by standard addition method in which pre analysed samples were taken and standard drug was added at three different levels. Values of recovery ± SD in the range of 98.0 % - 102 % indicate that proposed method is accurate for the analysis of the drug.

The precision of the proposed method was estimated in terms of inter-day precision and intra-day precision wherein the method was repeated on two different days and repeated for two different time periods in the same day respectively. The results shown in table 3 and 4 indicating %RSD less than 2% at each level clearly indicate that the proposed method is precise enough for the analysis of the drug.

Table 1: Recovery studies in 0.1 N HCl

Level	Pure drug conc. (µg/mL)	Cream conc. (µg/mL)	*Absorbance		Conc. found (µg/mL)		*Recovery (%) ± SD, % RSD	
			D^0	D^1	D^0	D^1	D^0	D^1
50%	2.5	5	0.396	0.018	7.52	7.42	101 ± 0.003, 0.75	99 ± 0.0003, 0.001
100%	5	5	0.520	0.025	10.11	9.92	102 ± 0.001, 0.19	99 ± 0.0005, 0.019
150%	7.5	5	0.645	0.125	12.51	12.42	100 ± 0.004, 0.62	98 ± 0.01, 0.01

* Mean recovery of three determinations

Table 2: Recovery studies in phosphate pH 2.0

Level	Pure drug conc. (µg/mL)	Cream conc. (µg/mL)	*Absorbance		Conc. found (µg/mL)		*Recovery (%) ±SD, %RSD	
			D^0	D^1	D^0	D^1	D^0	D^1
50%	2.5	5	0.48	0.020	7.6	7.4	101 ± 0.01, 1.0	98 ± 0.001, 0.05
100%	2.5	5	0.62	0.025	10.0	9.92	99.9 ± 0.02, 0.9	99 ± 0.0005, 0.02
150%	2.5	5	0.79	0.031	12.55	12.42	101 ± 0.01, 1.2	98 ± 0.0005, 0.0006

* Mean recovery of three determinations

Table 3. Results of method precision in 0.1 N HCl

Conc. (µg/mL)	INTRA DAY				INTER DAY			
	*Absorbance		*Assay (% w/w) ± SD, % RSD		*Absorbance		*Assay (% w/w) ± SD, % RSD	
	D^0	D^1	D^0	D^1	D^0	D^1	D^0	D^1
5	0.260	0.011	99.99 ± 0.02, 0.39	100 ± 0.02, 0.39	0.263	0.010	99.99 ± 0.02, 0.39	99.99 ± 0.02, 0.39
10	0.579	0.024	102 ± 0.06, 0.53	101 ± 0.05, 0.49	0.580	0.021	102 ± 0.06, 0.53	101 ± 0.05, 0.49
15	0.881	0.035	101 ± 0.05, 0.38	100 ± 0.04, 0.26	0.890	0.032	101 ± 0.04, 0.26	100 ± 0.04, 0.26

* Mean of three determinations

The method was applied to understand the stability aspects of the drug in a variety of stress conditions. The drug responded to the base stress in 0.1N HCl, base and photolytic stress in phosphate pH 2.0. which could be predicted from

the abnormal change in the assay values. The data is mentioned in table 5 and 6, the spectra are given in figure 6, 7 and 8.

Table 4: Results of method precision in phosphate pH 2.0

Conc. (µg/mL)	INTRA DAY				INTER DAY			
	*Absorbance		*Assay (w/w) ± SD, % RSD		*Absorbance		*Assay (w/w) ± SD, % RSD	
	D^0	D^1	D^0	D^1	D^0	D^1	D^0	D^1
5	0.275	0.013	99.99 ± 0.06, 0.53	99.99 ± 0.02, 0.39	0.278	0.012	99.99 ± 0.02, 0.39	99.99 ± 0.03, 0.43
10	0.555	0.025	102 ± 0.02, 0.39	102 ± 0.05, 0.49	0.552	0.024	102 ± 0.06, 0.53	101 ± 0.04, 0.54
15	0.831	0.033	101 ± 0.04, 0.26	101 ± 0.04, 0.26	0.830	0.032	100 ± 0.04, 0.26	101 ± 0.05, 0.43

* Mean of three determinations

Table 5: Stress degradation studies in 0.1N HCl

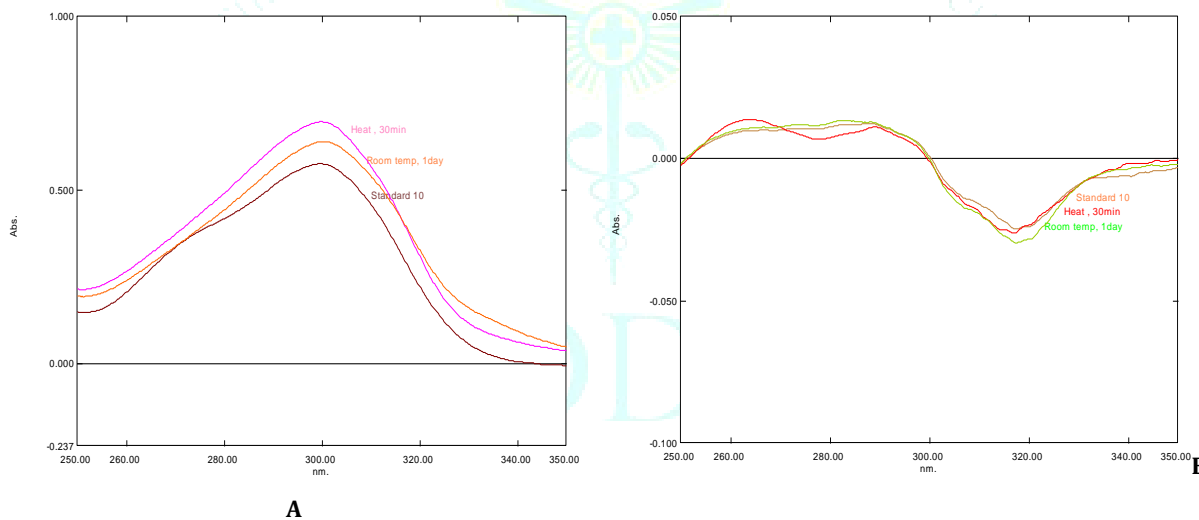| Conc. (µg/mL)	Condition	Absorbance		*Assay (w/w) ± SD, %RSD	
		D^0	D^1	D^0	D^1
10	Acid	0.577	0.023	102 ± 0.081; 0.12	100 ± 0.05; 0.49
	Acid heat	0.561	0.025	101.6 ± 0.002; 0.35	102 ± 0.08; 0.51
10	Base	0.663	0.024	120 ± 0.001; 0.15	101 ± 0.04; 0.54
	Base heat	0.605	0.029	109 ± 0.003; 0.23	104 ± 0.06; 0.61
10	Oxidation	0.542	0.024	100 ± 0.002; 0.20	101 ± 0.04; 0.54
	Oxidation heat	0.593	0.025	102 ± 0.004; 0.43	102 ± 0.08; 0.51
10	Thermal	0.542	0.024	100 ± 0.001; 0.29	101 ± 0.04; 0.54
	Thermal(solid)	0.560	0.023	101 ± 0.002; 0.36	100 ± 0.05; 0.49
10	Photo	0.503	0.020	98 ± 0.003; 0.13	98 ± 0.03; 0.13
	Photo (solid)	0.541	0.023	100 ± 0.002; 0.16	100 ± 0.05; 0.49

* Mean of three determinations

Figure 6: Base degradation spectra in zero order (A) and first derivative (B) (0.1N HCl)

Table 6: Stress degradation studies of phosphate pH 2.0

Conc. (µg/mL)	Condition	*Absorbance		*Assay (w/w) ± SD %RSD	
		D^0	D^1	D^0	D^1
10	Acid	0.534	0.022	100 ± 0.09; 0.12	99 ± 0.03; 0.38
	Acid heat	0.543	0.023	100 ± 0.005; 0.62	100 ± 0.05; 0.49
10	Base	0.139	0.004	25 ± 0.002; 0.75	21 ± 0.001; 0.22
	Base heat	0.693	0.029	126 ± 0.008; 0.9	104 ± 0.06; 0.61
10	Oxidation	0.555	0.023	101 ± 0.001; 0.21	100 ± 0.05; 0.49
	Oxidation heat	0.572	0.025	102 ± 0.002; 0.15	102 ± 0.08; 0.51
10	Thermal	0.566	0.024	102 ± 0.004; 0.17	101 ± 0.04; 0.54
	Thermal(solid)	0.562	0.024	102 ± 0.006; 0.20	101 ± 0.04; 0.54
10	Photo	0.638	0.030	114 ± 0.009; 0.15	106 ± 0.09; 0.63
	Photo (solid)	0.482	0.023	87 ± 0.002; 0.38	100 ± 0.05; 0.49

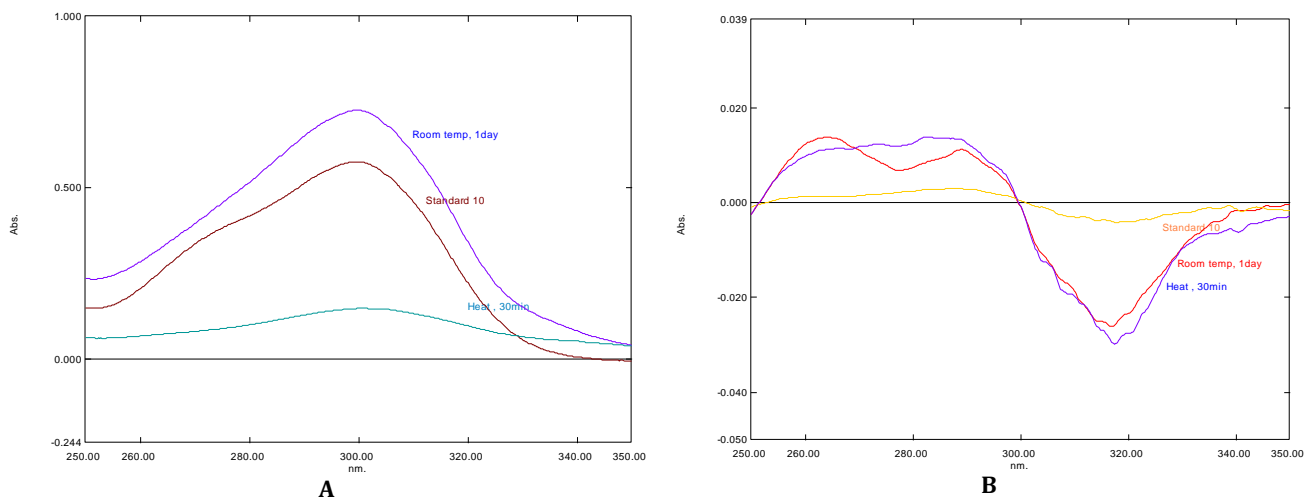* Mean of three determinations

Figure 7: Base degradation spectra in zero order (A) and first derivative (B) (Phosphate pH 2.0)
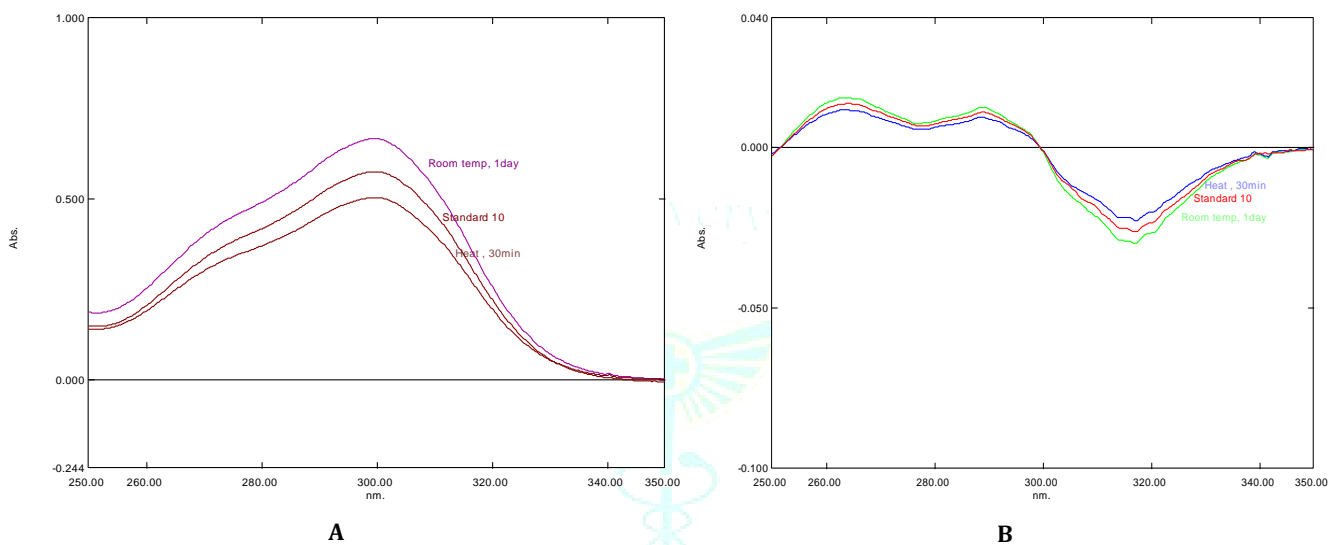

Figure 8: Photo degradation spectra in zero order (A) and first derivative (B) (Phosphate pH 2.0)

The developed methods were applied for the quantification of Luliconazole in creams and the assay obtained was within the limits which shows there is no interference from the excipients. The spectra are given in figure 9.
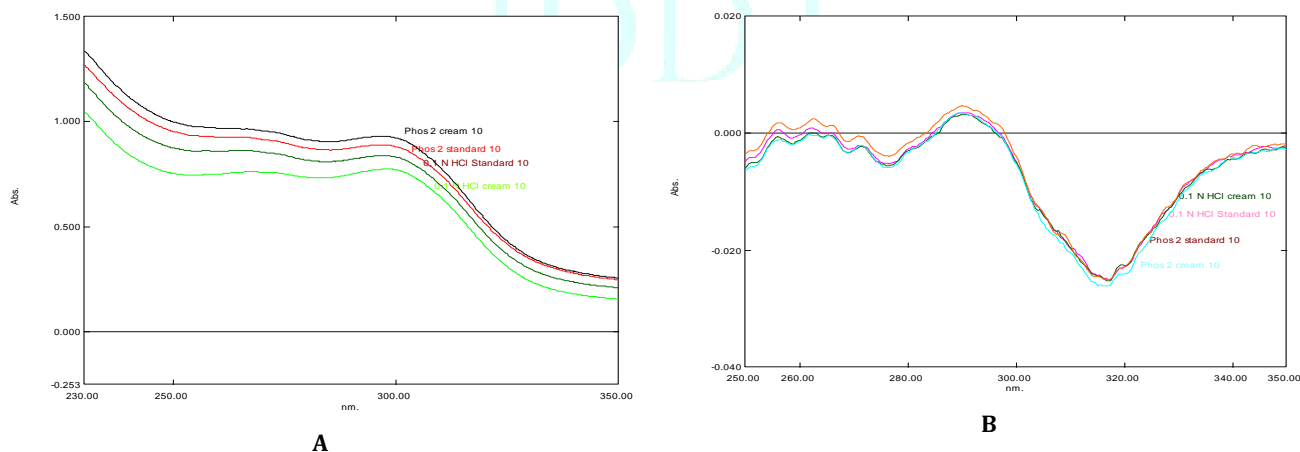

Figure 9: Overlain spectra for Assay in zero order (A) and first order derivative (B)

Table 7: Assay of formulation

Formulation	Label claim (% w/w)	*Amount obtained (% w/w)	*Assay ± SD
LULIFIN Cream (by sun pharma)	1	0.98	98.5 ± 0.85

* Mean of three determinations

Table 8: Optical characteristics and validation parameter of Luliconazole

PARAMETERS	OBTAINED VALUES			
	0.1N HCl	0.1N HCl	Phosphate buffer, pH 2.0	Phosphate buffer, pH 2.0
	D^0	D^1	D^0	D^1
λ_{max}/Minima, nm	294	315	294	317
Beer's Law limit (µg/mL)	2-30µg/mL	2-30µg/mL	10-30µg/mL	10- 30µg/mL
Sandell's sensitivity ($\mu g/cm^2$/0.001 AU)	0.016	-	0.017	-
Molar extinction Coefficient ($L.mole^{-1}.cm^{-1}$)	17700	-	17700	-
Regression equation (y=mx+c)*	0.0504x+0.0102	0.0025x+0.0002	0.0637x+0.0181	0.0025x+0.0006
Correlation co-efficient, R^2	0.9991	0.9991	0.999	0.999
LOD (µg/mL)	0.01	-	0.01	-
LOQ (µg/mL)	0.03	-	0.04	-

CONCLUSION

Two simple, precise and accurate UV spectroscopic methods methods have been proposed for the estimation of Luliconazole in bulk. These methods were found to be economical and could also predict the stability aspects of the drug in a broader sense and so can be conveniently used for the routine analysis of Luliconazole in dosage form.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

The authors have no conflict of interest.

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