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Research Article

Development and Validation of UV Spectrophotometric Method for the Estimation of Luliconazole in Bulk, Marketed Formulations.

Mohd Sayeed Shaikh[1], Mayura A. Kale*[,1], P. R. Mahaparle[1], Hrishikesh Rajput[2], Somnath M. Karkhele[1]

[1]Department of Pharmaceutical chemistry, Government College of Pharmacy, Osmanpura, Aurangabad-431 005, Maharashtra, India.

[2]Department of Pharmaceutical Chemistry, Shreeyash Institute of Pharmaceutical Education and Research, Aurangabad, Maharashtra, India.

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***Corresponding author E-mail address:** *kale_mayura@yahoo.com*

ABSTRACT

A UV spectrophotometric method was developed and validated for the determination of luliconazole (LZL) in bulk and cream formulation using a solvent composed of methanol: water (80:20) at a pre-determined λ_{max} of 298 nm, it was demonstrated linear in the range of 1.0–10.0 µg/mL, and exhibited good correlation coefficient (r^2=0.999) and excellent mean recovery (99.03–100.40 %). This method was successfully applied to the determination of LZL content in five marketed brands with recovery range (97.70–99.60 %) and the promising results obtained according to the label claims. The method was validated statistically and by recovery studies for linearity, precision, repeatability, and reproducibility. The method is also adopted for measurement of equilibrium solubility as per WHO guideline in different organic solvents and water. Method was also utilized for stability of analytical solution or short-term stability at room temperature (T_{RT}) and cold temperature (T_{CT})upto 72 hours as a part of robustness as per ICH Q2R1.Similarity Factor (S_f) and System Suitability Testing were found to be 1 &2.0 % respectively.

KEYWORD

Luliconazole, UV estimation, equilibrium solubility, stability of analytical solution, sf\Similarity Factor, System Suitability Testing.

1. INTRODUCTION

Luliconazole iodide (LZL) is a novel, broad-spectrum imidazole ring containing antifungal drug classified as Azoles and responsible for cell wall lysis by inhibiting lanosterol 14α-demethylase an enzyme responsible for synthesis of ergosterol in fungi. [1,2] US FDA approved LZL in the November 2013 for tineapedis and other dermal infections. It Stand in market with trade names Luzu, Lulicon U.S. Patents by Valeant Pharmaceuticals North America LLC. Luzu Cream (1%) indicated for the topical treatment of tinea incognito, tineapedis, tineacruris, and tineacorporis caused by the organisms *Trichophyton rubrum, Epidermophyton floccosum* and *Microsporum gypseum* [3–7] and also approved in Japan for the treatment of superficial mycosis, such as dermatophytosis, candidiasis, and pityriasis versicolor. [8,9] The MIC of LZL (MIC90) has been found to be 4 to 1,000-fold lower than the MIC90s reported for antifungals, including terbinafine, bifonazole, lanoconazole [1,10-11] and this is because of LZL is belongs Class-II of the BCS pharmaceutical classification.

Recently a few HPLC assay methods have been reported for the estimating of LZL. Literature survey revealed that some of the analytical methods such as stability-indicating assay for LZL in bulk and cream formulation on HPLC utilizing solvent system methanol and water (80:20, v/v); [12,13] RP-HPLC method has been developed for assay, related substances and validated for quantification of LZL in Topical Dosage form. [14] The mobile phase has been used for separation consisting of ammonium acetate buffer: ACN (60:40);[14] Tambe S. R.et al developed HPTLC method and validated for quantitative determination of LZL in pharmaceutical dosage form and biological fluid. [2] The R_f value of the drug was 0.62 ± 0.05 using Toluene: Methanol: Ethyl Acetate (6:2.5:0.5 v/v/v) as the mobile phase at 300 nm^2 and Leahy, M. K.et al has developed the LC-MS/MS method for the determination of the antifungal LZL in human toenails.[15]

In this research we have developed method which was optimized and validated as per the ICH guidelines Q2 (R1), WHO guideline July 2018 (QAS/17.699/Rev.2) and demonstrated excellent specificity, linearity, precision and accuracy for estimation of LZL in bulk and five marketed formulations. [16] Developed method was utilized for measurements of stability of analytical solutions as unit of robustness under the Q2R1 which was subjected to Bracketing and Matrixing designs as per (Q1d).

2. MATERIALS AND METHODS

2.1. Instrument (U V Apparatus)

A Shimadzu UV–visible spectrophotometer by UV -1700, Shimadzu Corporation, Kyoto, Japan was used for measurements and assessment of all absorbance and thereby related parameters with matched quartz cells.

2.2. Materials

All chemicals and reagents used for method development were of analytical or HPLC grade. LZL API chemically, (E)-2-((S)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene)-2-(1H-imidazol-1-yl)in the form of LZL iodide powder was provided by the Macleods Pharmaceuticals

Ltd. (Mumbai, Maharashtra, India) as the gift sample which was used as the reference standard. LZL cream (1%) of five different brands was selected from market for analysis and method development.

2.3. Determination of wavelength (λ_{max}) for maximum absorption

A standard stock solution (L_{ST}) of LZL (100 µg/mL) was prepared using diluents Methanol and Water in ratio 8:2. The maximum concentration solution for linearity range prepared by 1 mL of L_{st} was then diluted to 10 mL with the same diluents to obtain 1 µg/mL LZL reference solutions (L_{RF}). An UV spectroscopic scanning in photometric mode (200–400 nm) was carried out with the L_{RF} to determine the λ_{max} for the detection of LZL using diluents as blank in same ratio 8:2 figure 2.

2.3.1. Linearity and range

For linearity study, five solutions at different concentrations 0, 2, 4, 6, 8 and 10µg/mL were prepared using five different aliquots of L_{st} and the obtained data were used for the linearity study of LZL.

2.3.2. Intra-day precision (repeatability) and inter-day precision study (intermediate precision)

LZL creams (1%) of 10 grams of five different brand were extracted by using the liquid/solid extraction using methanol and water as the solvent and the sample stock solutions (L_{MST}) of 100 µg/mL was prepared following the same dilution pattern of L_{st}. five different aliquots of L_{PS} were then diluted to 10 mL to obtain the concentrations of 2, 4, 6, 8, and 10µg/mL.

2.3.3. Method Accuracy

This study was carried out using pre-formulated 1 % cream containing pure LZL and common excipients. Calculation was done from the label claim and the average weight of the final product. Previously used dilution pattern was followed for the 1 % cream to obtain five concentrations such as 80%, 90%, 100%, 110% and 120% of reference solution (L_{RF}).

2.3.4. Specificity in the presence of excipients

The test for the specificity was carried out using only excipients. Spectra for placebo cream, blank, and sample were compared.

Secondly the specificity was determined by subjecting the sample solution to accelerated degradation by heat (60º C) for 72 h in order to verify that none of their degradation products interfered with the quantification of the drug.

2.4. Assay of content of Luliconazole in selected marketed brands

Five market brands of LZL (1% w/w) cream from India were randomly selected and analyzed using the newly developed and validated method. Marketed sample standard stock solution (L_{MST}) 100 µg/mL is prepared. 1 gram of cream was taken into 100 ml volumetric flask and was diluted upto the mark with methanol and water (100 µg/ml). The solution was filtered through whatmann filter paper no.42. 1 mL of L_{MST} diluted to 10 mL to obtain 10 µg/mL LZL sample solutions of each brand (10 µg/mL) were prepared and assayed for content of LZL against the reference standard. Simultaneously 1 mL of L_{ST} diluted to 10 mL to obtain 10µg/mL LZL reference standard solution was also prepared and assayed for content of LZL against the

reference standard. The content of LZL in the marketed brands was determined using following equation no. 1.

Equation 1: (%) Content of Luliconazole in 1% cream of 10 gram

$$(\%)\ \boldsymbol{Content\ of\ luliconazole\ in\ 1\%\ cream\ of\ 10\ gram} = \frac{(As-Aif)}{Ast} \times \frac{Wst \times 1}{100 \times 10} \times \frac{100 \times 10}{Ws \times 1} \times W \times \frac{P}{100} \times CF$$ **---Equation 1**

where *As* is the absorbance of generic sample solution, Aif is the absorbance of interfering factors i.e. placebo cream has been prepared in lab as per respective marketed formulation formula, *Ast* is the absorbance of LZL reference standard solution, *Ws* is the weight of generic sample powder (mg), *Wst* is the weight of LZL reference standard powder (mg), *W* average net fill of the cream tube triplicates of each brand, *P* is the potency of standard LZL iodide and *CF* is the conversion factor of LZL API to LZL salt equal to 1 because both std and formulations contains salts.

2.5. Stability study

Samples prepared for repeatability study were preserved for 72 hours at T_{RT} & T_{CT} (2 – 8 ° C) then analyzed for three days as short-term stability test for solution stability.

2.6. Solubility studies of LZL in different solvents

Experiment design as per the WHO guideline July 2018 (QAS/17.699/Rev.2) to conduct equilibrium solubility of LZL for the purpose of prediction of solubility in different organic solvents and water. [17]

Solubility of LZL was determined by "shake flask" method as per WHO. The study was performed in triplicate by adding excess amount of drug to screw capped vials containing 2ml of distilled water and organic solvents individually. Then the vials were subjected to ultrasonication for 5 minutes thrice at an interval of 15min and then solubilized and mixed in vertex shaker to get uniform mixing until the saturation reached after the mixing allowed to stand at 37 °C +1 °C for 24 hours on orbital shaker with an optimized agitation rate without disturbance to attain saturation equilibrium. Filter the samples (using Whatmann filter paper no.41 / a filter pore size of 0.45 µ) immediately after withdrawal or separate dissolved from undissolved API by centrifugation as appropriate and analyzed spectrophotometrically at 298 nm after appropriate dilutions with methanol: distilled water (80:20) as per the above developed method. The absorbance of the samples was measured on UV spectrophotometer and content was recorded in Table 8.

3. RESULTS AND DISCUSSION

3.1. Method development and optimization

LZL is almost insoluble in aqueous medium and freely soluble in organic solvents like methanol, acetonitrile, DMSO, toluene, Chloroform and the solvents listed in solubility table. Hence the presented method is developed using methanol and water (80:20 v/v) as diluent. During the

development phase, the use of a few milliliters of methanol, acetonitrile, toluene and Chloroform with water as the diluent resulted in preferable outcome in UV analysis but the solvent system was optimized and utilized according to method developed by Sonawane S. P for HPLC estimation. It was observed that Luliconazole showed maximum absorption (λ_{max}) at 298 nm, hence the estimation was performed at λ_{max} 298 nm for all the measurements. [12,13]

3.2. Method validation

3.2.1. Similarity Factor (S_f)

Similarity Factor (S_f) was determined by the standard sampling method, comparing between two standards samples including bracketing sample done and it was found to be 1. A_{std} weight of LZL taken and A1 and A2 solutions where made and respective absorbance was measured at 298 nm. Similarly, B_{std} weight of LZL taken and B1 to B6 solutions prepared and analyzed according to needs of ICH. The similarity factor found within the standard range 0.99 – 1.0. Similarity factor adopted by different regulatory body viz. US-FDA / MHRA / WHO as criteria of system suitability for any analytical instrument. The S_f was determined by following equation no. 2.

Equation 2: Similarity Factor (S_f)

$$Sf = \frac{Wt\ A_{std}}{Wt\ B_{std}} \times \frac{Abs\ (B1-B6)_{avg}}{Abs\ (A1-A2)_{avg}}$$ **---Equation 2**

Table 1. Determined of Similarity Factor (S_f) by the standard sampling method.

S. No.	Sampling	Absorbance
1	A1	0.768
2	A2	0.768
3	B1	0.769
4	B2	0.769
5	B3	0.769
6	B4	0.769
7	B5	0.768
8	B6	0.768
12	B-BKT	0.768
9	AVG (B-BKT, B2-B6)	0.769
10	SD	0.001
11	(%) RSD	0.07

3.2.2. System Suitability Testing

System suitability was determined by three replicate sampling of the system suitability solution. The acceptance criteria are relative standard deviation of the absorbance of standard solutions should not be more than 2.0 %. The absorbance of highest concentration should not be greater than1 and not less than 0.1. The system suitability results obtained for LZL is summarized in

Table 1. It was observed that the method complies with system suitability parameters. Hence, the system suitability parameter meets the requirement of method linearity and range.

3.2.3. Validation

3.2.3.1. Linearity

The calibration curve obtained in spectrum mode after method development was evaluated by its correlation coefficient (r^2). The absorbance of the samples in the range of 2.0–10.0 µg/mL was linear with the r^2 greater than 0.999. The LOD and LOQ were calculated as 0.04µg/mL and 0.012µg/mL respectively.

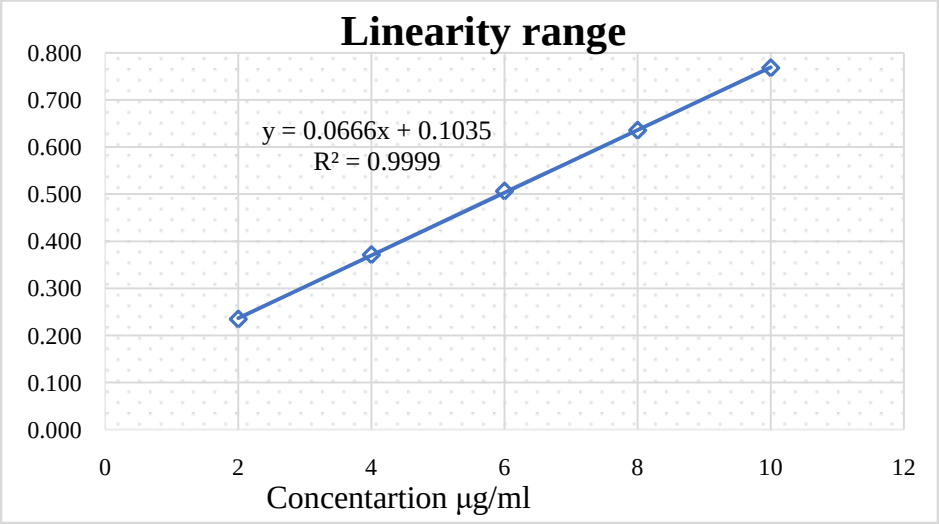

Fig. 1. Linearity range of LZL by proposed method.

3.2.3.2. Intra-day and inter-day precision studies

The intra-day and inter-day precision study as showed in Table3 of the developed method confirmed adequate sample stability and method reliability where all the RSDs were < 2%.

Table 2. Intra-day and inter-day precision determined for three different concentrations of LZL.

Sr. No.	**Drug Conc.**	**Intra-day Precision**			**Inter-day Precision**		
		Average Conc. (n=3)	**SD**	**%RSD**	**Average Conc. (n=3)**	**SD**	**%RSD**
1	4	3.98	0.002	0.563	3.88	0.008	2.1
2	4	4.02	0.002	0.469	3.93	0.008	2.231
3	4	3.99	0.001	0.312	3.94	0.007	1.953
4	6	6.03	0.002	0.303	5.94	0.004	0.718
5	6	6.01	0.002	0.303	5.9	0.004	0.806
6	6	5.97	0.005	0.939	5.92	0.004	0.795
7	8	7.97	0.002	0.241	7.95	0.002	0.329
8	8	7.94	0.001	0.157	7.93	0.002	0.315

9	8	7.92	0.002	0.242	7.97	0.002	0.33
Average RSD				0.392	Average RSD		1.064

3.2.3.3. Short term Stability / Solution stability

Solution Stability study results were within the acceptance range represented in table4 and indicated the samples solution stability over 72 hours (short-term) at both T_{RT} and T_{CT} in absorbance and linearity.

Table 3. Short term stability at room temperature (T_{RT}) and 2-8º C (T_{CT}).

Conc. declared µg/mL	**Room Temperature (T_{RT})**			**2-8º C (T_{CT})**		
	Average of Conc. found (Mean + SD, µg/mL)	**RSD (%)**	**Average potency (%)**	**Average of Conc. found (Mean + SD, µg/mL)**	**RSD (%)**	**Average potency (%)**
4	3.848 ± 0.001	0.0012	96.05	3.87 ± 0.002	0.002	96.77
6	5.910 ± 0.0006	0.0006	98.55	5.92 ± 0.001	0.001	98.81
8	7.950 ± 0.001	0.0012	99.43	7.96 ± 0.001	0.001	99.58

3.2.3.4. Accuracy/recovery

Results within the range of 99.03–100.40% ensure an accurate method given in table 5 as well as indicate non-interference with the excipients of formulation after extraction from the cream formulation.

Table 4. Recovery/accuracy for five different concentrations of LZL by the proposed method.

SR. No.	**Drug Conc.**	**Addition**	**Average**	**SD**	**%RSD**
1	4	3.2	7.166667	0.057735	0.805605
2	4	4	8.033333	0.057735	0.718693
3	4	4.8	8.766667	0.057735	0.658574
4	6	4.8	10.73333	0.11547	1.075808
5	6	6	11.96667	0.057735	0.482465
6	6	7.2	13.13333	0.057735	0.439607
7	8	6.4	14.36667	0.057735	0.401868
8	8	8	15.9	0.1	0.628931
9	8	9.6	17.53333	0.057735	0.329287
Average RSD					0.615649

3.2.3.5. Specificity in the presence of excipients/Accuracy

The specificity of the analytical method was proved by comparing the spectra of placebo and degradation product of sample solution with that of accuracy sample (Figure. 2).

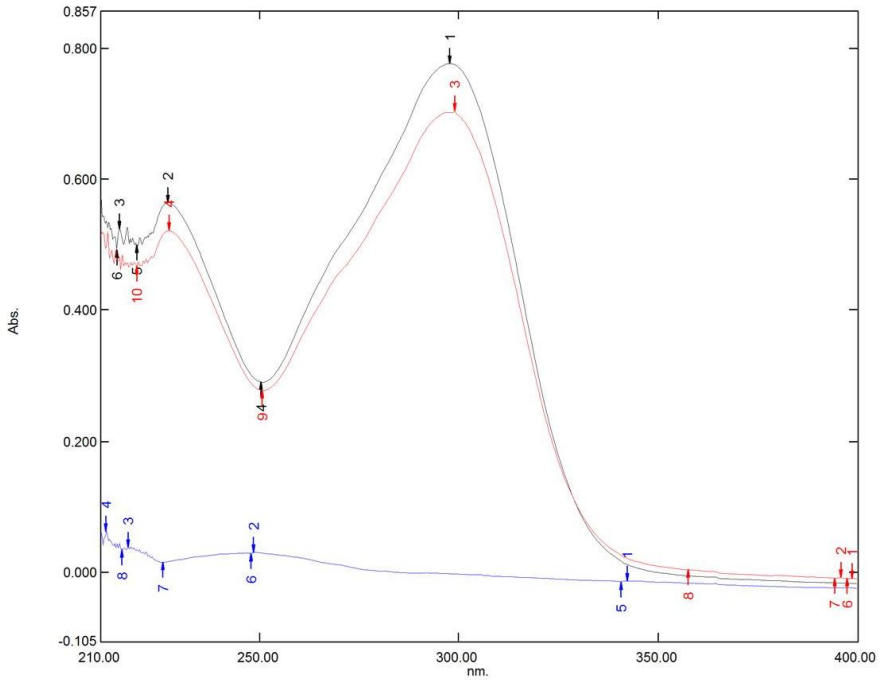

Fig. 2. Specificity of the method determined by comparing the spectra of accuracy sample (Purple), placebo (Blue) and degradation products (Red).

3.2.3.6. Content of LZL in marketed brands

LZL content of five marketed products determined by the proposed method (Table 4) was in good agreement with the label claims and was in the range of 97.70–99.60% with the RSD values of 0.076 –0.272 % respectively.

Table 5. Content of LZL in marketed brands.

Sr. No.	Marketed Cream	F1	F2	F3	AVG	SD	%RSD	ASSAY
1	LUL01	0.754	0.754	0.757	0.755	0.002	0.229	98.3%
2	LUL02	0.765	0.765	0.764	0.765	0.001	0.076	99.6%
3	LUL03	0.763	0.76	0.76	0.761	0.002	0.228	99.1%
4	LUL04	0.764	0.767	0.763	0.765	0.002	0.272	99.6%
5	LUL05	0.75	0.751	0.75	0.750	0.001	0.077	97.7%

3.3. Solubility studies of LZL in different solvents

The experimentation solubility studies for the purpose of classifying APIs within the BCS. The procedure adopted WHO guidance on equilibrium solubility experiments and solubility measurements included in the Brazilian Pharmacopoeia in 2016. The method is also adopted for measurement of solubility in different organic solvents and the results are presented in table 8. The results confirm that LZL is belongs to Class II drug as per BCS and practically insoluble in

water as per USP Solubility Criteria but soluble in organic solvents this indicates that LZL has high permeability due to lipophilicity and log P value. In case of LZL, solubility is an intrinsic properties that may be affected by solvent properties like relative polarity, Dipole movement and dielectric constant. As the relative polarity of solvent increases solubility of LZL decreases for example LZL is sparingly soluble in IPA which has relative polarity 0.546 and freely soluble & very soluble in ethyl acetate and Chloroform having relative polarity 0.228 and 0.259 respectively. Similarly solubility decreases as dipole movement of solvent decreases viz. diethyl ether and IPA with the dipole movement 1.15 and 1.66 is sparingly soluble as compare to the pyridine and DMSO having values 2.2 and 3.9 are very soluble and freely soluble. But all the above discussion is for the solubility is applicable to the organic solvents only.

Table 6. Equilibrium solubility of LZL in organic solvent and water.

Sr. no.	**solvents**	**Solubility (milligram/ml)**			**Solubility (gram/ml)**				**Solubility as per USP Solubility Criteria**	**relative polarity 2**	**Dipole moment**
		S1	**S2**	**S3**	**S1**	**S2**	**S3**	**Avg.**			
1	DMSO	785.1	798.2	791.2	0.7851	0.7982	0.7912	0.7915	Freely soluble	0.444	3.9
2	n-Butanol	66.79	67.54	65.97	0.06679	0.06754	0.06597	0.0668	soluble	0.586	1.7
3	Chloroform	2654	2627	2706	2.654	2.627	2.706	2.6623	Very soluble	0.259	1
4	Ethyl Acetate	410.7	414.1	395.8	0.4107	0.4141	0.3958	0.4069	Freely soluble	0.228	1.78
5	IPA	25.47	26.74	25.89	0.02547	0.02674	0.02589	0.0260	Sparingly soluble	0.546	1.66
6	Diethyl ether	22.46	22.32	24.18	0.02246	0.02232	0.02418	0.0230	Sparingly soluble	-	1.15
7	Pyridine	3142	3078	3193	3.142	3.078	3.193	3.1377	Very soluble	0.302	2.2
8	Ethanol	85.71	86.58	85.22	0.08571	0.08658	0.08522	0.0858	soluble	0.654	1.7
9	Methanol	691.2	693.2	684.2	0.6912	0.6932	0.6842	0.6895	Freely soluble	0.762	1.6
10	Acetic acid	905.2	898.6	911.2	0.9052	0.8986	0.9112	0.9050	Freely soluble	0.648	1.68
11	Benzene	934.2	943.1	948.6	0.9342	0.9431	0.9486	0.9420	Freely soluble	0.111	0
12	Aceton	221	213	219	2.213	2.162	2.194	2.189	Very soluble	0.355	2.8

	e	3	62	94				7			5
13	CCL_4	47.65	48.43	47.22	0.04765	0.04843	0.04722	0.0478	soluble	0.052	0
14	Acetonitrile	895.4	903.4	890.3	0.8954	0.9034	0.8903	0.8964	Freely soluble	0.46	3.5
15	NH_3	5.41	5.62	5.57	0.00541	0.00562	0.00557	0.0055	Slightly soluble	-	1.42
16	Toluene	3560	3611	3585	3.56	3.611	3.585	3.5853	Very soluble	0.099	0.36
17	Water	0.06326	0.0645	0.06227	6.326 x 10^{-5}	6.45 x 10^{-5}	6.27 x 10^{-5}	6.36 x 10^{-5}	Practically insoluble & insoluble	1	1.85
18	DMF	17.29	18.06	17.1	0.01729	0.01806	0.0171	0.0175	Sparingly soluble	0.386	3.86
19	Dioxane	3874	3795	3895	3.874	3.795	3.895	3.8547	Very soluble	0.614	0.45
20	propylene glycol	2645	2726	2604	2.645	2.726	2.604	2.6583	Very soluble	-	3.796

4. CONCLUSION

The present analytical method was developed and validated as per ICH Q2(R1) and WHO guidance on equilibrium solubility experiments and solubility measurements included in the Brazilian Pharmacopoeia in 2016 for BCS and Biowaiver study of LZL. Analytical method meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate, robust and having stability indicating characteristics. The Solvent system used for method development was methanol and water (80:20) and this solvent system was also stable for short term solution stability at TRT and TCT with good percent recovery. UV method development and solubility study given in this paper apply to APIs and conditions for dissolution studies applicable to finished solid pharmaceutical products (FPP).

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