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SIMULTANEOUS ESTIMATION OF LINAGLIPTIN AND RESVERATROL: DEVELOPMENT AND VALIDATION OF A UV-SPECTROSCOPIC STABILITY-INDICATING METHOD

Nazreen Tabassum[1], Rizwan Ahamad[2], Rama K P[1], Bushra Jabi[1], Pragati Kumari Gupta[3], Mohammad Vaseem Ismail[4], Mohd Mujeeb[5], Mohd Aqil[1]*

[1]Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi – 110062, India.
[2]Department of Pharmacology, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi – 110062, India.
[3]Department of Zoology, Department of Zoology, Maharani Janki Kunwar College, Bettiah, West Champaran, Bihar-845438, India.
[4]School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi– 110062, India.
[5]Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi – 110062, India.

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Abstract

The simultaneous identification and detection of both medications is a challenge when a pharmaceutical product is formulated with a dual or combination drug delivery system. The most straightforward, precise, and dependable technique among the many that are accessible is UV spectroscopy. We have successfully developed and validated the UV Spectroscopy technique for concurrent estimation of Linagliptin (LGT) and Resveratrol (RSV). The percentage recovery of each drug at 50%, 100%, and 150% of the developed simultaneous equation was found to be 50.3% to 49.7%, 100.8% to 101.3%, and 150.3% to 149.7%for LGT and RSV, respectively. The percent relative standard deviation of the LGT and RSV mixture, the intra-day analysis was determined and found to be 1.221% at λ_1 and 1.162% at λ_2, respectively. In the inter-day study, % RSD was determined to be 1.136% at λ_1 and 1.393% atλ_2, respectively. Based on the data, we conclude that our approach is exact and accurate. Through the use of forced deterioration testing in a stability study, we have further verified our approach. Both of our medications were shown to breakdown in oxidative, alkaline, and acidic degradation conditions. Thermal and photolytic degradation studies were also conducted. Based on the findings, it was determined that our simultaneous estimation UV Spectroscopy approach is dependable and can open up new research avenues.

***Keywords*:** UV spectroscopy, Force degradation study, Simultaneous estimation, Linagliptin, Resveratrol, Stability study.

***Corresponding Author**
Dr. Mohd Aqil

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INTRODUCTION

In the pharmaceutical development of a new formulation, it is essential to identify and measure the medication to optimize formulation parameters such as stability. Antioxidants can decrease or inactivate free radicals and/or reduce oxidative damage [1]. Our formulation comprises a dual medication system consisting of Linagliptin (LGT) and Resveratrol (RSV). Our future objective is to augment neurodegenerative potential through the combination of LGT and RSV, as both medicines possess the capacity to address anti-Alzheimer's activity along with anti-diabetic properties.

Linagliptin (LGT) (Fig. 1A), recognized chemically as (R) (2-ynyl-3-methyl-8-(3-aminopiperidin-1-yl)-7-but 1-(2-ylmethyl-4-methylquinazolin) -3,7 dihydro-purine 2,6-dione, is a reversible and competitive inhibitor of DPP-4 [2]. Linagliptin keeps the blood glucose level stable in diabetes patients by helping the pancreas produce more insulin and alerting liver to inhibit making glucose when blood sugar levels are high [3].

Resveratrol (RSV), a polyphenolic molecule found in berries, grapes, peanuts, and wine, is chemically characterized as 3,4′,5-trihydroxystilbene; mol. wt. 228.247 g/mol (Fig. 1B). Resveratrol has anti-inflammatory, anti-tumor, anti-apoptotic, and

antioxidant activities [4]. Furthermore, it has been shown that this natural chemical possesses neuroprotective properties.

Furthermore, research has shown that resveratrol can prevent damage to both neuronal and glial cells, control brain function, and enhance behavioural aspects related to memory, learning, anxiety, and depression [5]. The maximum amounts of resveratrol in the blood and brain can be seen 20–30 minutes after oral ingestion and can last for up to 60 minutes, according to recent studies. Because of this, resveratrol can penetrate blood-brain barrier (BBB), indicating cognitive effects.Since resveratrol has anti-inflammatory properties in addition to its possible impact on neurological illnesses, there is pharmacological interest in using it in these conditions [6].

Formulating a combination presents a problem for concurrent examination and recognition of both drugs utilized. Among the several methods for drug determination, spectrophotometry remains favored due to its simplicity, specificity, and cost-effectiveness [7]. Following a comprehensive literature review, it was noted that only isolated methodologies for the examination of both medicines are accessible.

An isosbestic point enables easy, accurate quantification and interpretation by providing a common wavelength for compounds with different absorption maxima [8]. Based on the isosbestic point, we have devised and validated for concurrent assessment of LGT and RSV using UV spectroscopy.

The developed approach is straightforward, sensitive, precise, and reproducible, which can be validated by assessing the stability of LGT and RSV under stressful conditions. Forced degradation testing of pharmaceutical compounds is mandated by International Conference on Harmonization (ICH) guidelines to ascertain the compound's intrinsic stability by delineating a degradation pathway and identifying probable degradation products [9]. This will demonstrate that the methodology utilized for the analysis is robust and effective. "Stability testing of innovative pharmaceutical compounds and products, in accordance with ICH standards Q1A (R2) and Q1B, must be performed under diverse conditions, encompassing extreme temperatures, oxidation, photolysis, and hydrolysis across a wide pH spectrum [10].

MATERIALS AND METHODS

1. Instruments

Shimadzu UV-1600 series equipment was utilized in conjunction with a quartz cuvette featuring a 1 cm path-length. Software utilized was UV Probe version 4.2. A computerized analytical balance was used in the experiment (Wenstar DA14-222) and an ultrasonic sonicator (Equitron). Micro pipettes, volumetric flasks, and beakers were constructed from borosilicate glass.

2. Chemicals and Reagents

Pure drug specimens of Linagliptin and Resveratrolhave been purchased from Yucca Enterprises, Mumbai, India. Methanol was purchased from Merck Limited, Mumbai. All reagents and chemicals were using this study AR Grade.

3. Selection of wavelength

In a 100ml standard volumetric flask, 10mg of precisely weighed LGT was solubilized in methanol, and volume was elevated to 100ml with methanol to attain a concentration of 100µg/ml. 1 ml of above stock solution was pipetted and put into a 10 ml standard volumetric flask, with volume adjusted to 10ml utilizing methanol to achieve aamount of 10µg/ml. Above solution was subsequently analyzed between 200-400 nm against a blank sample.

Similarly, a 100ml standard volumetric flask was filled with 5 mg of RSV that had been carefully weighed and solubilized in methanol. Volume was then adjusted to 50ml using methanol to reach a concentration of 100µg/ml. 1ml of aforementioned stock solution was pipetted and put into a 10ml standard flask, and volume was modified to 10ml with methanol to achieve a conc. of 10µg/ml. The aforementioned solution was analyzed within the 200-400 nm range against the reagent blank.

4. Concurrent development and validation of LGT and RSV in methanol via UV spectroscopy

The absorbance of two drug samples was measured at their respective maximum absorption wavelengths for the quantitative analysis of two components using the simultaneous equation method. The absorbances of LGT and RSV were measured at selected wavelengths of 233 nm (λ1) and 306 nm (λ2), respectively. The absorptivity coefficients for each drug were determined at both wavelengths. Absorbance and absorptivity coefficients were correctly replaced into the equations, resulting in the determination of the concentration values of each drug in the mixture. Concentrations of two drugs in a 2:1 mixture at 233 nm and 306 nm were determined utilizing the following equation:

$$C_x = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{ax_1} \ldots \text{eq. (1)}$$

$$C_Y = \frac{Q_M - Q_X}{Q_Y - Q_X} \times \frac{A_2}{ay_1} \ldots \text{eq. (2)}$$

Where, A1 and A2 are the aggregate absorbance at 233 and 306 nm, respectively.

ax1= A (Absorptivity, 1 %, 1 cm) of LGT at 233 nm (53.2)

ay1= A (Absorptivity,1 %, 1 cm) of RSV at 233 nm (126.4)

ax2= A (Absorptivity,1 %, 1 cm) of LGT at 306 nm (69.7)

ay2= A (Absorptivity,1 %, 1 cm) of RSV at 306 nm (170.4);

Cx and Cy represent unidentified concentrations of LGT and RSV, correspondingly, in specimen solution.

QM= A2/A1, QX= ax2/ ax1 and QY= ay2/ay1…eq. (3)

The combination's absorbance at 233 and 306 nm is indicated by A1 and A2, respectively; absorptivities of LGT at λ1 and λ2 are represented by ax1 and ax2, while absorptivities of RSV at λ1 and λ2 are represented by ay1 and ay2. The LGT and RSV concentrations are shown by Cx and Cy, respectively.
At 233 nm, solutions of both drugs at varying concentrations exhibited similar absorbance, indicating no change. Wavelengths exhibiting similar isosbestic or iso-absorptive points are absorptivity for two specie [11,12].

5. Preparation of standard stock solution

Precisely measured quantities of 10mg of LGT and 5mg of RSV were individually placed into a 100 ml volumes flask. Initially, approximately 50ml of methanol was added into flask and subjected to sonication. Volume was modified to indicate with methanol to make stock solutions of 100μg/ml of LGT and RSV. Subsequently, a requisite quantity of this stock solution was diluted with methanol to produce conventional solutions varying from 2.5 to 12.5μg/ml for LGT and 2 to 10μg/ml for RSV. Absorbance of resultant solutions was quantified at peak absorption wavelengths of 233 and 306 nm, respectively.

6. Preparation of test solution for assay

Weighed approximately 10 mg of LGT and 5 mg ofRSV, dissolve them in methanol in a 100ml standard volumes flask, and adjust volume to 100ml and 50ml with methanol to attain a level of 100μg/ml. Whatman filter paper no. 41 was utilized to filter mixture. From stock solution given earlier, 1ml was pipetted and transferred into a 10ml standard volumetric flask, and volume was modified to 10ml with methanol to achieve aamount of 10μg/ml. Solution was analyzed within spectrum of 200-400nm in comparison to reagent blank.

7. Preparation of calibration curve

Aliquots of 0.25, 0.5, 0.75, 1.0,and 1.25ml were precisely transferred from working standard solutions of LGT (100μg/ml) into a series of 10 ml volumetric flasks using a validated 1ml pipette. Each flask was then diluted to mark with methanol using a validated 10ml pipette. The results produced levels of 2.5, 5, 7.5, 10, and 12.5μg/ml of LGT.
Using a validated 1 ml pipette, aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml were precisely transferred from working solutions of RSV (100μg/ml) into a series of 10ml volumetric flasks. Each flask was then diluted to mark with methanol using a validated 10ml pipette. The results produced concentrations of 2, 4, 6, 8, and 10μg/ml of RSV. Absorbance of resulting solutions was assessed at maximum absorption wavelengths of 233 and 306 nm, respectively.

8. Method Validation as per ICH guidelines Q2 (R1)

The proposed technique has been validated in compliance with ICH guidelines Q2 (R1) [11].

9. Linearity and Range

Study of linearity involved the preparation of standard solutions at five distinct amounts. Linearity range for LGT and RSV was established as 0-12.5 μg/ml and 0-10 μg/ml, correspondingly. Absorbance of LGT and RSV was quantified at λ1 and λ2 for each solution. Calibration curves depicting absorbance vs concentration were established. By using linear regression analysis, a linear connection between absorbance responses and concentrations was discovered. Experiment was conducted in triplicate [13].

10. Precision

Accuracy of suggested approach was examined by repeatability, intra-day precision, and inter-day precision. Repeatability was assessed by doing five replicates of specimen solution. Intermediate precision was assessed by assessing five replicates' reactions on same and distinct days for a test solution that containsLGT and RSV at a level of 10μg/ml and 5μg/ml. The results were expressed as a % RSD [13].

11. Accuracy

Recovery investigations were conducted using the conventional addition approach. A specified quantity of standard LGT and RSV (5, 10, and 15μg/ml), corresponding to 50%, 100%, and 150% oflabel claim, was included into a test solution of LGT and RSV (10μg/ml). The identical investigation was conducted thrice, at each stage of recovery.13

12. Detection and quantitation limits

Detection limit (DL) is minimal concentration of the analyte which could be accurately identified, reflecting the precision of the instrumental response. Quantitation limit (QL) denotes the minimal amount of substance in a specimen that could be measured with adequate precision and accuracy. QL and DL were calculated utilizing equations (4) and (5), which are based on standard deviation and slope of acquired response. Where σ denotes standard deviation of intercept of calibration plot and S signifies slope of calibration curve [13].
Limit of Detection (LOD) and Limit of Quantification (LOQ) of new technique were found from calibration curve utilizing formula,

$$\mathrm{LOD} = 3.3 \times \sigma/S \quad \ldots\text{eq. (4)}$$

$$\mathrm{LOQ} = 10 \times \sigma/S \quad \ldots\text{eq. (5)}$$

Where, σ represents standard deviation of y-intercepts of regression lines of calibration curves, and S is average of slopes of calibration curves.

13. Forced degradation studies

Forced degradation experiments of LGT and RSV were conducted to evaluate the efficacy of the analytical

approach. To establish a stability-indicating test method, UV-spectrophotometry in methanol was employed. Stability indicating UV-spectrophotometric assay technique for the simultaneous assessment of LGT and RSV was carried out first time [3,14].

14. Sample preparation for forced degradation

LGT and RSV were subjected to degradation under various circumstances, such as acidic, basic, oxidative, thermal, and photolytic environments. Subsequent degrading techniques employed were:

15. Acidic and alkaline degradation

From working solution, 1 ml of LGT and 2 ml of RSV were placed into separate 10 ml flasks for degradation tests. After adding 3 ml of 0.1N HCl to LGT and RSV flasks for acidic conditions, same procedure was performed with 0.1N NaOH for alkaline conditions. Following that, volumetric flasks were kept at 70°C in dark reflux for 30 minutes for acidic and alkaline conditions. Diluents were used to increase volume to 10ml to measure absorbance.

16. Oxidative and thermal degradation

From working solution, transfer 1ml of LGT and 2 ml of RSV into a separate flask, and to each 1ml of 6 % w/v of H2O2was added, and volume was modified to 10 ml indicate with diluent. Then, volumetric flasks were kept in dark for 15 min, and absorbance was taken. A thermalanalysis was conducted by taking 1ml of LGT and 2 ml of RSV from working solution into separate 10ml flask. Then kept in hot air oven at 90°C for 1 hr and volume was adjusted to 10ml and absorbance was determined.

17. Photolytic degradation

LGT and RSV samples were exposed to direct sunlight for 4 hrs. 1 mg of each specimen was placed in separate 10ml volumetric flasks, solubilized in methanol, and volume was adjusted to 10 ml to provide a concentration of 10μg/ml. The sample was subsequently positioned in a cuvette for UV analysis [3,14].

RESULTS AND DISCUSSION

This section presents the diverse findings of our testing, demonstrating an acceptable UV spectrophotometric method applicable at various stages of the study. Solutions of LGT and RSV, with a level of 10μg/ml and 5μg/ml, were successfully produced independently and scanned within the 200-400 nm range. The absorbance ratio technique employs ratio of absorbance at two specific wavelengths, one of which is λmax of one of two components and other is an iso-absorptive point. The overlay spectra of both drugs indicate that LGT and RSV possess an iso-absorptive point at 233 nm (λ1). Second wavelength utilized was 306 nm (λ2) of λmax of RSV.LGT and RSV exhibited significant absorption at both wavelengths, as depicted in Fig 02.

1. Design and evaluation of a UV spectroscopic approach for concurrentassessment of LGT and RSV in methanol

LGT and RSV samples were analyzed at their corresponding observed maximum absorption wavelengths, i.e., 233 nm (λ1) and 306 nm (λ2), correspondingly. Absorptivity coefficients of LGT and RSV were computed at wavelengths of 233 nm (λ1) and 306 nm (λ2) individually and are presented in Table 1. This was executed in triplicate. Absorptivities of LGT at λ1 and λ2 were determined to be 53.2 (ax1) and 69.7 (ax2), accordingly. Similarly, absorptivities of RSV at λ1 and λ2 were determined to be 126.4 (ay1) and 170.4 (ay2), respectively. Absorbance of mixture of solution was found to be 0.350 (A1) and 0.201 (A2) for LGT and RSV respectively.

The concentrations of drugs Cx and Cy in the combination, determined by applying the absorbance and absorptivity coefficients to the specified equations, were determined to be -2.40μg/ml and 1.53μg/ml, correspondingly. Total absorbance of LGT and RSV solution is equal to the mixture of LGT and RSV solution absorbance value, here Q absorption method was mathematically proven in methanol.

2. Linearity and range

A calibration curve was established. The absorbance v/s concentration of the standard was graphed, and the regression equations were derived as illustrated in Fig. 3 and 4 for LGT and Fig. 05 and 06 for RSV, respectively. Additional parameters were calculated as detailed in Tables 01 and 02.

3. Precision

The %RSD of repeatability, intraday and inter-day precision was determined for λ1 and λ2, respectively, as presented in Table 04.

4. Accuracy as recovery

The % recovery of LGT and RSVwas determined at 50%, 100% and 150% varying from 50.3 % to 49.7 %, 100.8 % to 101.3 % and 150.3 % to 150.7%respectively, as presented in Table 05.

5. Forced degradation studies

LGT and RSV underwent degradation at various circumstances, such as acidic, basic, oxidative, thermal, and photolytic environments. Table 06 presents the results of the deterioration analysis. Both LGT and RSV were significantly destroyed in acidic and basic media, as well as during oxidative degradation. However, there is minimal deterioration under photolytic and thermal conditions, as showed in Table 06.

Table 01: Linearity data of LGT at 233 & 306 nm.

S. No.	LGT at λ1 233 nm			LGT at λ2 306 nm		
	Concentration (µg/ml)	Absorbance*±SD	% RSD	Concentration (µg/ml)	Absorbance*±SD	% RSD
0	0	0	0	0	**0**	**0**
1	2.5	0.204±0.001	0.746	2.5	0.097±0.001	1.569
2	5	0.413±0.001	0.370	5	0.173±0.001	0.578
3	7.5	0.619±0.001	0.247	7.5	0.259±0.001	0.589
4	10	0.834±0.001	0.120	10	0.352±0.001	0.433
5	12.5	0.986±0.001	0.155	12.5	0.441±0.001	0.346
* Average of 6 ascertainment. (SD= Standard deviation, % RSD = Relative standard-deviation).						

Table 02: Linearity data of RSV at 233 & 306 nm.

S. No	RSV at λ1 233 nm			RSV at λ2 306 nm		
	Concentration (µg/ml)	Absorbance*±SD	% RSD	Concentration (µg/ml)	Absorbance*±SD	% RSD
0	0	0	0	0	0	0
1	2	0.176±0.001	0.568	2	0.215±0.003	1.395
2	4	0.352±0.003	0.866	4	0.417±0.001	0.366
3	6	0.523±0.001	0.292	6	0.637±0.002	0.314
4	8	0.709±0.001	0.215	8	0.872±0.001	0.175
5	10	0.876±0.001	0.174	10	0.985±0.002	0.203
* Average of 6 as certainment. (SD= Standard deviation, % RSD = Relative standard deviation).						

Table 03: Attributes determined for UV spectrophotometric method.

Parameters	Drugs			
	LGT		RSV	
Beer's Law limit (µg/ml)	2.5-12.5 µg/ml		2-10 µg/ml	
λ max	233 nm	306 nm	233 nm	306 nm
Correlation Coefficient (r^2)	0.9981	0.9992	0.9999	0.998
Slope	0.0803	0.035	0.0879	0.0108
Intercept	0.0078	0.0021	0.0002	0.005
SE of Intercept	0.16722	0.10997	0.03251	0.003868238
SD of Intercept	0.40954	0.26933	0.07964	0.009473315
LOD (µg/ml)	16.83	25.39	2.99	19.22
LOQ (µg/ml)	51.00	76.95	9.06	58.26

Table 04: Repeatability, intraday, and inter-day precision of LGT and RSV.

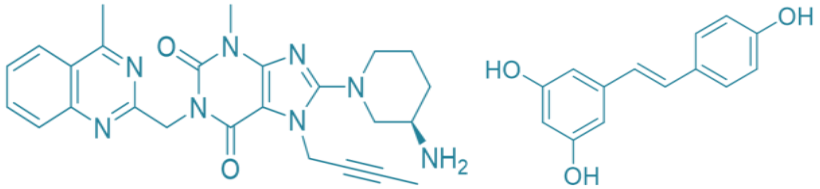
	Concentration of sample		Absorbance*±SD		% RSD	
	LGT	RSV	233 nm	306 nm	233 nm	306 nm
Inter-day Precision	10	5	0.097±0.001	0.116±0.002	1.136	1.393
Intraday Precision	10	5	0.091±0.001	0.114±0.001	1.221	1.162
Repeatability	10	5	0.095±0.001	0.118±0.002	0.875	1.27

Table 05: % recovery of LGT and RSV.

	Drug	Average (Absorbance of 3)	± SD	% Recovery	Avg ± SD	% RSD
50 %	LGT	5.03	0.058	50.3	5.03±0.058	1.147
	RSV	4.97	0.058	49.7	4.97±0.058	1.1625
100%	LGT	10.07	0.166	100.8	10.07±0.166	1.649
	RSV	10.13	0.057	101.3	10.13±0.057	0.569
150%	LGT	15.03	0.208	150.3	15.03±0.208	1.3847
	RSV	14.97	0.058	149.7	14.97±0.058	0.3858

Table 06: Results of forced degradation studies of LGT and RSV by UV spectrophotometry.

S. No.	Forced Degradation Conditions		% Concentration recovered		% Degradation Observed	
			Linagliptin	Resveratrol	Linagliptin	Resveratrol
1	Acid Hydrolysis	0.1N HCL (70˚C, 30 min)	83.23	67.23	16.76	32.76
2	Alkali Hydrolysis	0.1N NaOH (70˚C, 30 min)	76.89	61.62	23.10	38.37
3	Oxidative Degradation	6% w/v H2O2 (Room temp., 15 min)	85.56	82.74	14.43	17.25
4	Thermal Degradation	90˚C for 1 hour in a hot air oven	81.36	82.46	18.63	17.53
5	Photolytic Degradation	4 hrs of exposure to direct sunlight	78.64	79.39	21.35	20.60



(A) (B)

Fig. 01: Chemical structures of Linagliptin (A) and Resveratrol (B)

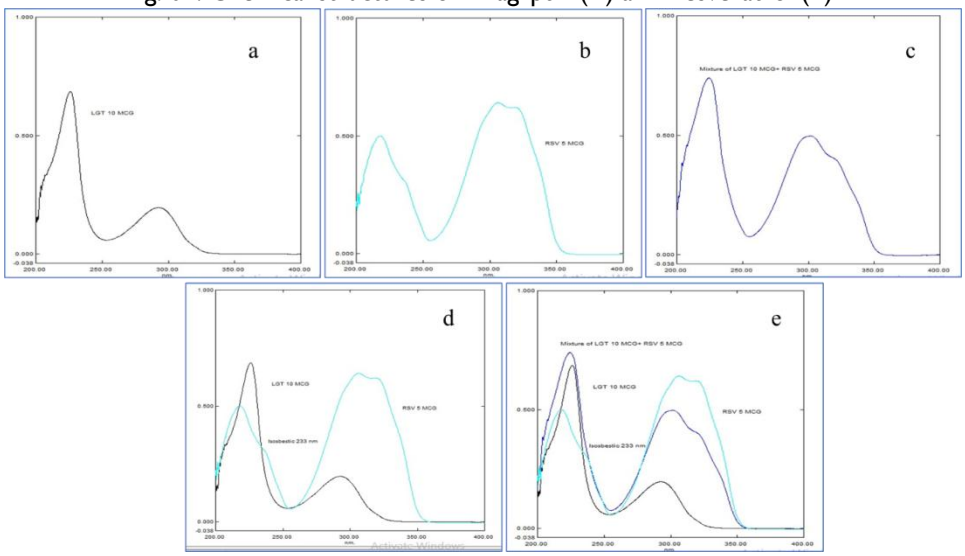


Fig. 02: Determination of λmax of LGT (a), RSV(b), Physical mixture of LGT and RSV(c), Overlay spectra of LGT and RSV in methanol illustrating their iso-absorptive point at 233 nm (d), Overlay spectra of LGT, RSV, and Physical mixture of both the drugs in 2:1 ratio (e).

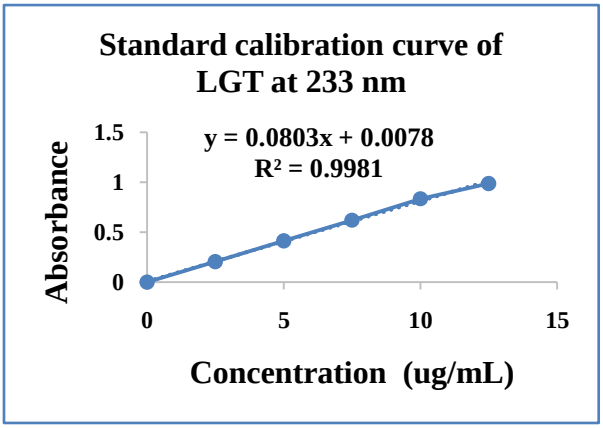


Fig. 03 Curve of calibration of LGT at λ_1 = 233 nm

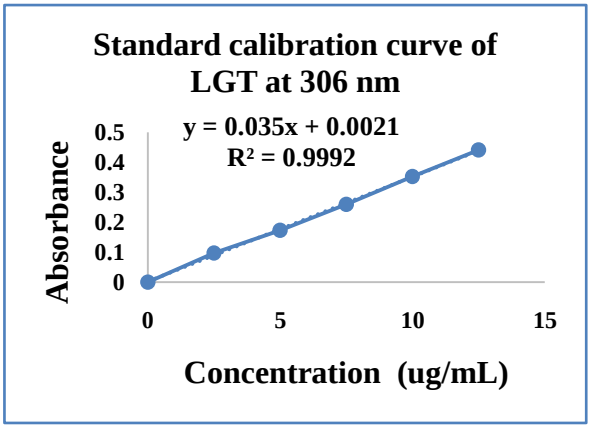


Fig. 04 Curve of calibration LGT at λ_1 = 306 nm

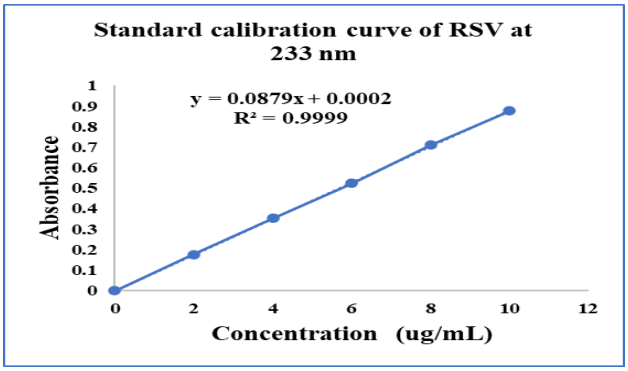


Fig. 05: Curve of calibration of RSV at λ = 233 nm

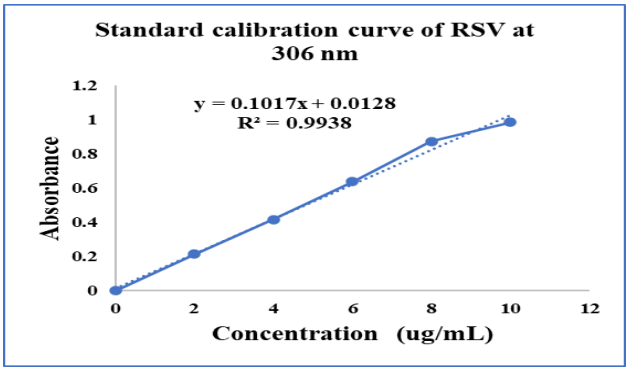


Fig. 06 Curve of calibration of RSV at λ_1 = 306 nm

CONCLUSION

We have successfully developed and validated a method for concurrent assessment of LGT and RSV in combination formulation using methanol as the solvent. The results are suitable with acceptance parameters in compliance with ICH guidelines, which are linearity, accuracy, precision, LOD and LOQ. Forced degradation research serves as an indicator of a drug's stability under stressed or extreme settings, indicating its effectiveness in detecting degradation products without interference from active ingredients which is essential for industry and academic research. Consequently, it is essential to comprehend a drug substance's purity profile and its behavior under diverse environmental conditions. Therefore, we ascertain that our newly developed UV spectrophotometric approach is a dependable indication of stability and may be employed for stability examination.

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AUTHOR CONTRIBUTIONS

Nazreen Tabassum: Conceived and designed the experiments; Nazreen Tabassum, Rizwan Ahamad, Rama K.P. Performed the experiments; Nazreen Tabassum, Rizwan Ahamad: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools, or data; Bushra Jabi, Pragati Kumari Gupta, and Mohammad Vaseem Ismail: Writing, reviewing, and editing the manuscript. Mohd Mujeeb and Mohd Aqil: Analyzed the data and supervised the research work.

DATA AVAILABILITY STATEMENT

Data are available on request from the corresponding author.

CONFLICT OF INTREST

The authors declare that they have no conflicts of interest.

INFORM CONSNET AND ETHICAL STATEMNET

Not Applicable

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