IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2022 Vol.:24, Issue:1

Method Development and Validation of Stability Indicating UV Visible Spectrophotometric Method for Levonorgestrel in Bulk and Tablet Dosage Form

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ISSN 2349-7203
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Sudha.T*, Vetrichelven.T [1], Nivetha. S[2], Pavithra.V[3], Priyadharshini.B[4], Priyanka.V[5]

*[*1, 2, 3, 4,5] Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur – 603319, Tamilnadu, India.*

Submitted: 25 March 2022
Accepted: 31 March 2022
Published: 30 April 2022

Keywords: Analytical method, Levonorgestrel, Degradation, UV Spectroscopy, Validation

ABSTRACT

A simple, specific, accurate, and stability-indicating UV-Spectrophotometric method was developed for the estimation of levonorgestrel, using a Lab India UV-3000 plus double beam. Methanol was used as a solvent. Linearity was established for Levonorgestrel in the range of 15-75µg/ml. The percentage recovery of levonorgestrel was found to be in the range of 99.93-100.08%. The drug was subjected to acid, alkali, oxidation, and photolytic degradation study. Validation experiments were performed to demonstrate specificity, precision, linearity, accuracy, LOD, and LOQ. While determining the marketed formulation there was no interference of excipients and other additives. The LOD and LOQ value was found to be 0.0707µg/ml and 0.2142µg/ml. Hence this method can be used for routine determination of levonorgestrel in bulk and the pharmaceutical dosage form. The proposed method for stability studies shows that there was appreciable degradation found in stress conditions of levonorgestrel.

INTRODUCTION

Levonorgestrel ($C_{21}H_{28}O_2$) is a synthetic oral contraceptive drug. Levonorgestrel (or norgestrel or d-norgestrel chemically known as 13β-ethyl-17β-hydroxyl-18,19-dinor-17αpregn-4-en-20-yn-3-one (fig.1). It is second-generation synthetic progesterone used as an active ingredient in some hormonal contraceptives [1]. It is also used as a single agent in emergency contraception and has hormonal contraceptives released from an intrauterine device. Levonorgestrel prevents pregnancy by interfering with ovulation, fertilization and implantation. By binding with progesterone and androgen receptors in the hypothalamus it slows down the release of the gonadotropin-releasing hormone as a result suppression of luteinizing hormone takes place which inhibits the rupture of follicles and viable egg release from the ovaries.[2-3]

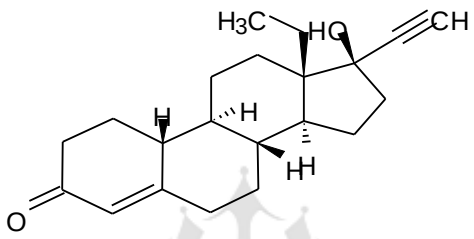

Fig. No. 1: Structure of Levonorgestrel

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in a solution. The instrument which measures the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region, is called Ultraviolet-Visible spectrophotometers.

Stability testing forms a heavy part in the process of pharmaceutical product development. Stability testing aims to provide evidence and how the quality of drug substances varies at different times under the influence of various environmental factors. Stability testing by forced degradation involves the degradation of drug substances under the influence of different accelerated conditions like Acid, Base, Oxidation and Light. [4].

On reviewing the literature survey for the determination of levonorgestrel by RP-HPLC [5, 6], LC-MS/MS [7-8], LC-MS [9-10], and UPLC-MS/MS [11-12] methods were reported. Levonorgestrel combined with other drugs by using HPLC [13], RP-HPLC [14-17], and UPLC-MS [18] methods also were reported. No method has been reported for the determination of

levonorgestrel by stability indicated using UV–Visible Spectrophotometry. Hence, the present study aims to develop and validate the stability-indicating UV-Visible Spectrophotometric method for the estimation of Levonorgestrel in bulk and tablet dosage form.

MATERIALS AND METHODS

UV-Visible spectra were recorded on a Lab India UV-3000 plus double beam spectrophotometer using 1.0cm quartz cells. All weighing was done on Shimadzu's electrical balance. Bath sonicator was used to aid dissolution. Glassware was calibrated for each step. Pure drug samples of levonorgestrel were obtained from Madras Pharmaceuticals, Chennai.

All other chemical reagents used were of analytical grade. Methanol, sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) was purchased from Loba chemicals. A locally available brand (Piramal 1.5mg) of levonorgestrel has been purchased from the local pharmacy to conduct an assay with the developed method.

Selection of solvent and λ max

About 10mg of levonorgestrel are treated with different solvents such as distilled water, sodium hydroxide, hydrochloric acid, methanol, ethanol, chloroform, ethyl acetate, petroleum ether, acetonitrile, and acetone and di-methyl Sulphoxide were added to determine the solubility. From the solubility study, Methanol was selected as a solvent because levonorgestrel was soluble in methanol. 30µg/ml of stock solution was prepared to determine the maximum wavelength (λmax). The solution was scanned between the range of 200-400 nm in a UV-visible spectrophotometer. The λmax of levonorgestrel was found to be 240nm.

Preparation of standard stock solution

About 15mg of levonorgestrel was accurately weighed and transferred into a 100ml volumetric flask and the volume was made up to the mark by using methanol as a solvent, which contains (150µg/ml). From this further dilution was carried out in 1ml to 10ml. Finally, the concentration of the solution was 15µg/ml.

Linearity

150µg/ml standard stock solution was prepared. From the standard stock solution, 1, 2, 3, 4, 5 ml were pipetted out separately and transferred into a separate 10ml standard flask. Then the

solution was made upto the mark with methanol. The final concentration 15, 30, 45, 60 and 75 µg/ml was obtained.

Quantification of raw material

About 30µg/ml of standard solution was prepared and measured the absorbance at 240nm. The amount of raw material was found by using slope and intercept values from the reports to check the purity of the standards (API).

Assay

An assay (content estimation) was performed to determine the purity of Levonorgestrel in tablet formulation. The nominal concentration from the calibration curve was selected and quantification of Levonorgestrel was performed. The tablet formulation Piramal (1.5 mg) was selected for analysis.

Precision

The precision of the method was confirmed by intermediate precision analysis. The analysis was carried out by using a sample solution (30µg/ml). The analysis was done for six times.

Accuracy

The accuracy of the method was evaluated by, a known amount of pure drug concentrations 50%, 100%, and 150% were added to the previously analyzed solution of formulation and the mixture was analyzed by the proposed method. %RSD values were calculated. The %RSD values were found to be less than 2%. Hence it indicated that there was no inference due to excipients containing formulation.

Degradation study

Levonorgestrel API was undergone forced degradation study under acid and base hydrolysis as well as oxidative, photolytic stress conditions. Only thermal degradation of drug substance was carried out in solid-state. Solutions were prepared by dissolving drug substances in a solvent methanol to obtain a concentration of 1000µg/ml and later diluted with the solvent methanol to achieve the approximate concentration of 10µg/ml. Finally, a spectral scan ranges from 200-400nm in UV/Vis spectrophotometer was performed to take absorbance after 24 hours for observing the degradation study.

Acid hydrolysis of drug substance in solution state was conducted with 0.1N HCl at room temperature for 24 hours. Base hydrolysis of drug substance in solution state was conducted with 0.1N NaOH solution at room temperature for 24 hours. For oxidative stress, sample solutions of drug substance in 0.1% H_2O_2 were kept at room temperature for 24 hours. For thermal stress, solid samples of drug substances and drug products were kept at sunlight for 24 hours.

RESULTS AND DISCUSSION

The drug was identified by IR spectroscopy. IR interpretation report was shown in table 1. In IR interpretation the following major peaks are 891 cm^{-1} and 691cm^{-1} indicating =C-H out of plane bending confirm the presence of an aromatic ring, 1651 cm^{-1} indicated C=C stretching confirms the presence of aromatic ring,1681cm^{-1} indicated C=O stretching confirm the presence of two conjugated aromatic ring and ketone, 2932 cm^{-1} indicated C- H and O-H stretching confirm the presence of methylene, 3268cm^{-1} indicated O-H bond confirm the presence of alcohol and 3340cm^{-1} indicated ≡C-H stretching confirms the presence of alkyne[19]. Hence, the IR data showed confirmed the drug was Levonorgesterol. The IR spectrum was shown in figure 2.

TABLE NO. 1: IR Interpretation Data of Levonorgestrel

S.NO	FREQUENCY	WAVENUMBER	FUNCTIONAL GROUPS
1.	3340	≡C-H Stretch	Alkynes
2.	3268	O-H	Alcohol
3.	2932	C-H stretching	Methylene
4.	2853	O-H Stretching	Aldehyde hydrogen
5.	1651	C-H Stretching	Aromatic
6.	1618	C=C Stretching	Conjugated and ketones and 2-aromatic ring
7.	891	C=O	Aromatic ring
8.	691	Stretching =C-H out of the plane	Aromatic ring

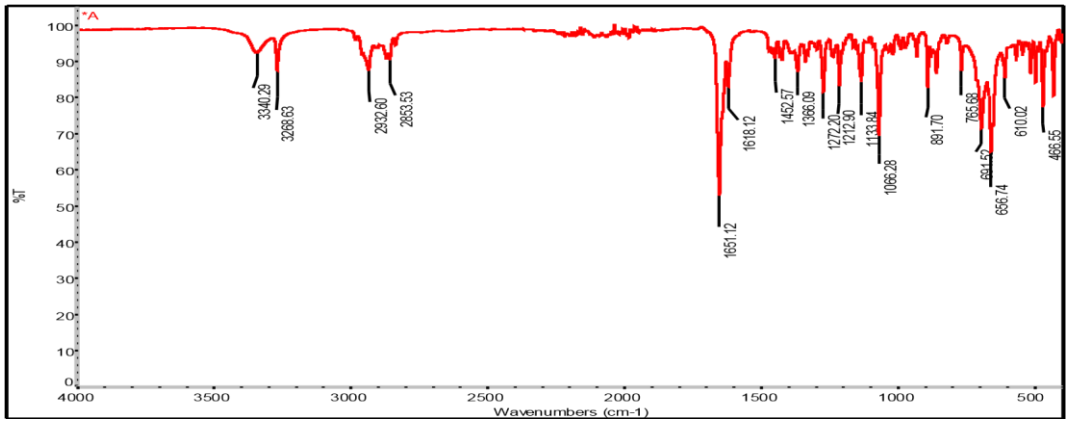

Fig. No. 2: IR Spectrum of Levonorgestrel

30µg/ml concentration solutions of Levonorgestrel were prepared and the spectrum was recorded. The spectrum was shown in figure 3a at 240 nm wavelengths by observing the spectral characters of Levonorgestrel. The overlay spectrum was shown in figure 3b.

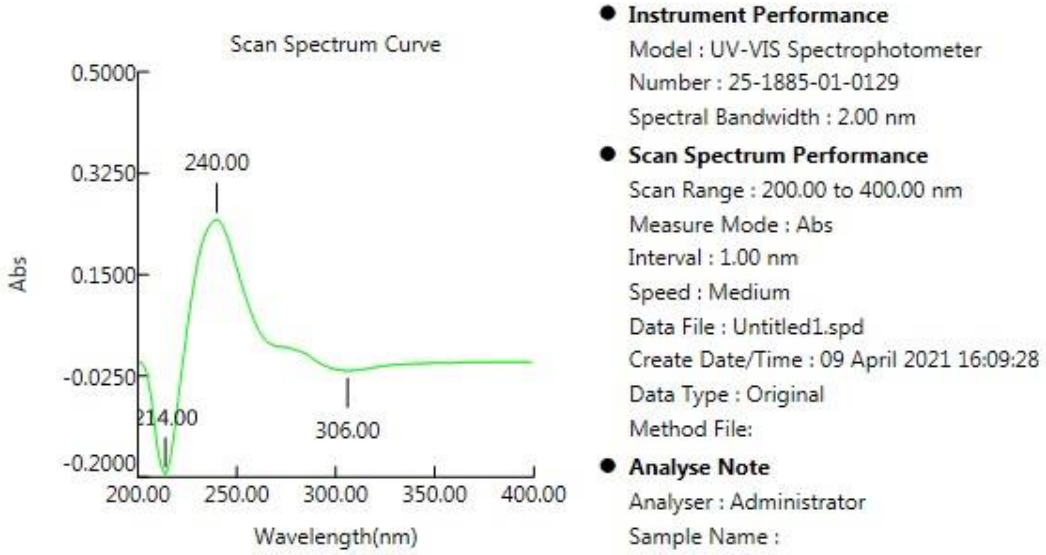

Fig. No. 3a: UV SPECTRUM OF LEVONORGESTREL

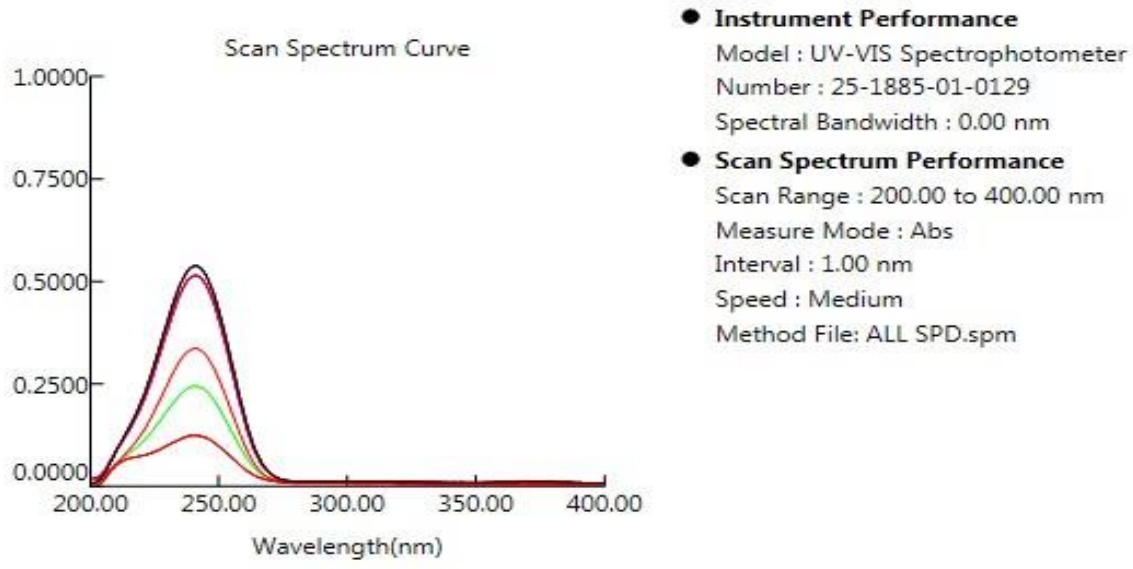

Fig. No. 3b: OVERLAY SPECTRUM OF LEVONORGESTREL

The described method has been validated as per ICH guidelines [20]. The proposed method was found to be linear with a linear correlation coefficient of 0.999 and the linear regression Equation, Y= 0.014X+0.0026. The calibration curve was shown in figure 4. The minimum concentration levels at which levonorgestrel can be reliably detected (LOD) and quantified (LOQ) were found to be 0.0707 and 0.2142 µg/ml, respectively.

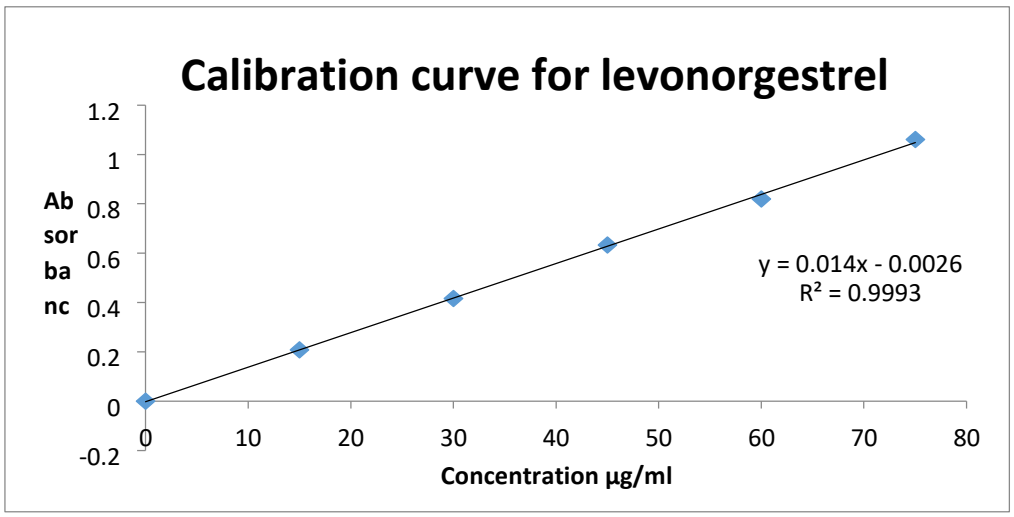

Fig. No. 4: CALIBRATION CURVE FOR LEVONORGESTREL

The optical parameters report were shown in table 2. The LOD and LOQ value was very less. Hence, it indicated the method was very sensitive. Quantification of raw material was done. The nominal concentration of the standard solution was approximately 30µg/ml. The reports of analysis were shown in table 3. The mean percentage purity of the tablet was found to be 99.78. The report of analysis was shown in table 4.

TABLE NO. 2: Optical Parameters of Levonorgestrel

PARAMETERS	**At 240nm**
Beer's law	15-75µg/ml
Correlation coefficient	0.9993
Regression equation	Y=0.014x-0.0026
Slope	0.014
Intercept	0.0026
LOD(µg/ml)	0.0707(µg/ml)
LOQ(µg/ml)	0.2142(µg/ml)

TABLE NO. 3: Quantification of Levonorgestrel

Drug Name	**Sample No**	**Concentration (µg/ml)**	**Amount found (µg/ml)**	**Average (%)**	**SD**	**% RSD**
Levonorgestrel	1	30	30.9	99.86	0.7684	0.7694
	2	30	31.0			
	3	30	30.7			
	4	30	30.2			
	5	30	30.6			
	6	30	30.1			

TABLE NO. 4: Assay of Levonorgestrel Tablet formulation

Drug Name	Sample No	Concentration (µg/ml)	Amount found (µg/ml)	Average (%)	SD	%RSD
Levonorgestrel (Piramal 1.5mg)	1	30	30.9	99.78	0.7259	0.7275
	2	30	31.0			
	3	30	30.6			
	4	30	31.2			
	5	30	31.2			
	6	30	30.7			

The precision of the method was confirmed by intermediate precision. % RSD value was found to be less than 2%. So the method was found to be precise. The results were shown in table 5. The accuracy of the method was analyzed by using the standard addition method. The % recovery was found to be in the range of 99.93 to 100.08 and the %RSD value was found to be 0.0814. The low %RSD (less than 2%) value indicated that the method was accurate and no interference due to excipients. The results were shown in table 6.

TABLE NO. 5: PRECISION DATA FOR LEVONORGESTREL FORMULATION

Drug Name	Sample Number	Concentration (µg/ml)	Amount found (µg/ml)	Percentage obtained (%)	Average (%)	SD	%RSD
Levonorgestrel	1	30	30.85	99.47	100.01	0.5985	0.5984
	2	30	31.00	99.95			
	3	30	31.14	100.40			
	4	30	30.78	99.24			
	5	30	31.07	100.18			
	6	30	31.28	100.86			

TABLE NO. 6: Accuracy of Levonorgestrel

Concentration (%)	Amount present (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	SD	%RSD
50	30.0	15.0	45.02	15.02	100.06		
100	30.0	30.0	59.98	29.98	99.93	0.0814	0.0814
150	30.0	45.0	75.04	45.04	100.08		

The study was performed to validate the stability-indicating capability of the developed method and to identify the key factors, which will impact the stability of the drug. The specificity was determined according to ICH guidelines by subjecting a standard solution to various stress conditions like acid, base hydrolysis, oxidative and photolytic conditions [20].

The degradation was remarked for acid hydrolysis of levonorgestrel standard when react with 0.1N HCl at room temperature, for 24 hours. The degradation amount of levonorgestrel standard was found to be 63.22%. The degradation was remarked for base hydrolysis of levonorgestrel standard when react with 0.1N NaOH at room temperature, for 24 hours was found to be 80.4%. The degradation was remarked for oxidation of levonorgestrel standard when react with 0.1% H_2O_2 at room temperature, for 24 hours, was found to be 36.5%. The degradation was remarked for photolytic degradation of levonorgestrel when react with sunlight and the degradation was found to be 133.17%. The stability testing data represented the % degradation was found to be more than 20% was shown in table 7, which does not meet the acceptance criteria (% limit of degradation 5-20%) So the drug levonorgestrel was not stable under the mentioned stress conditions acid, base, oxidative and photolytic. The different stress conditions spectrum was shown in figure 5a-d.

TABLE NO. 7: Degradation Data of Levonorgestrel

S. NO.	Stress condition	% Degradation
1.	Acid 0.1 N HCl	63.22
2.	Alkaline 0.1N NaOH	80.4
3.	Oxidative 0.1% H_2O_2	36.5
4.	Photolytic degradation	133.17

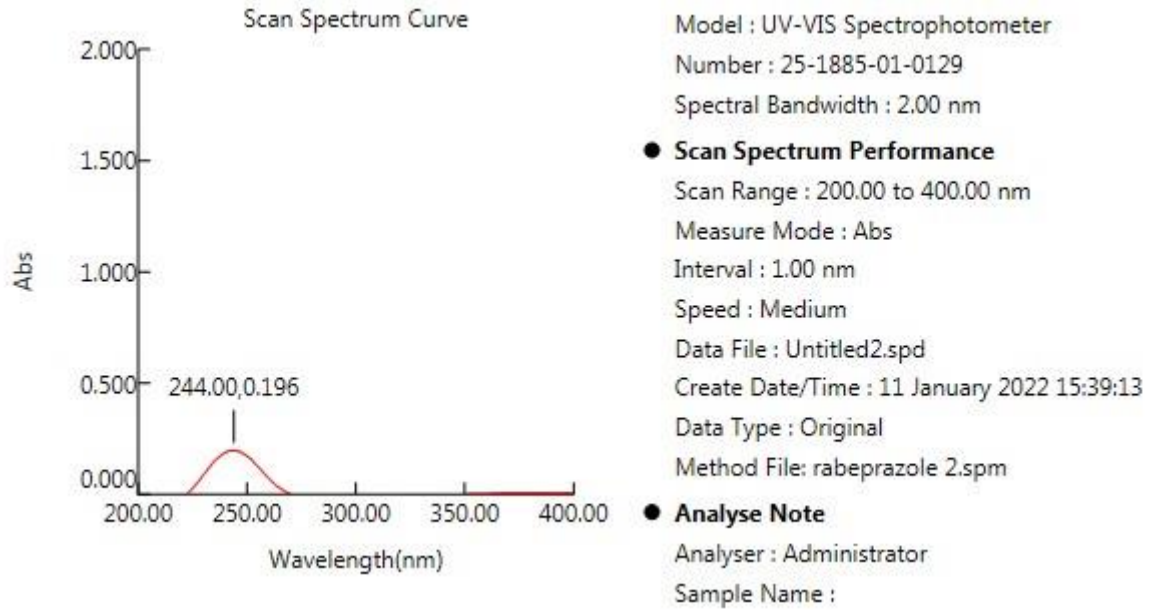

Fig. No. 5a: DEGRADATION SPECTRUM FOR 0.1N HCl

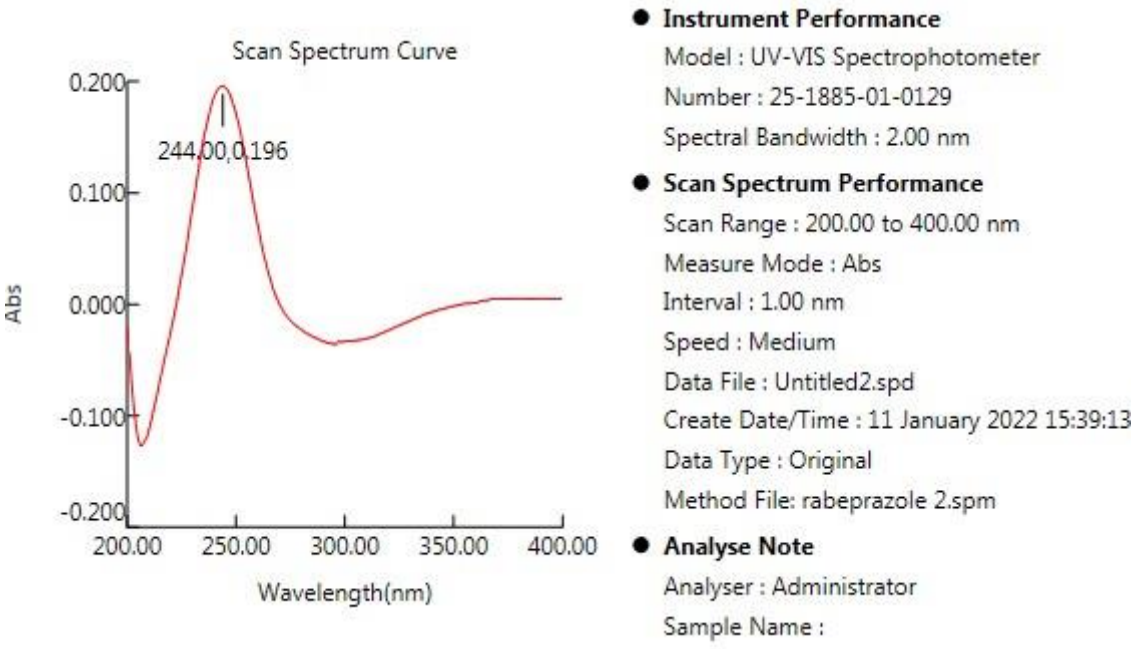

Fig. No. 5b: DEGRADATION SPECTRUM FOR 0.1N NaOH

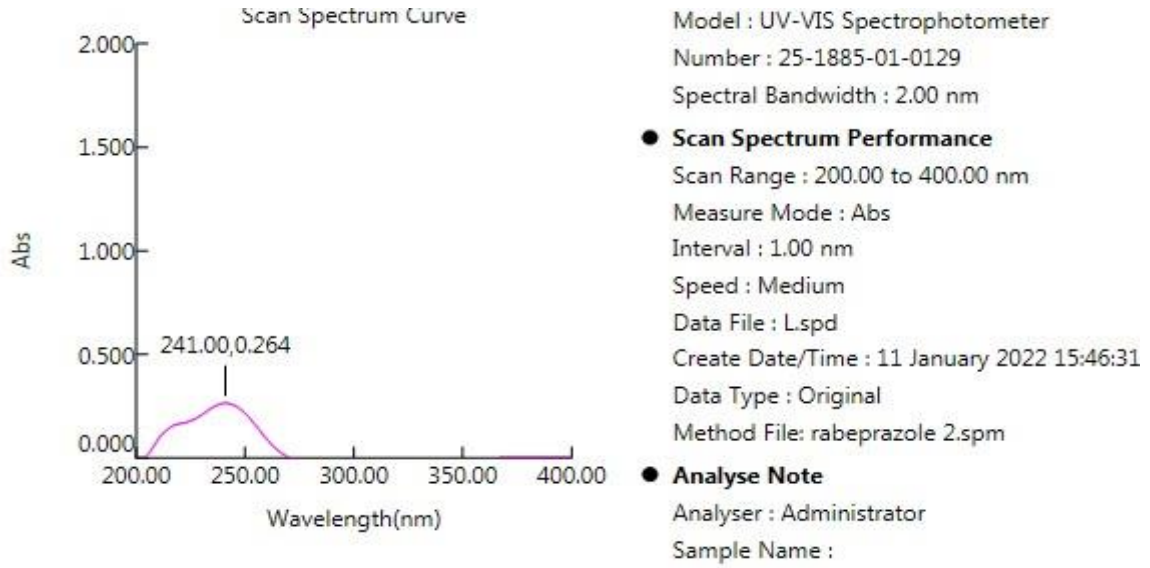

Fig. No. 5c: DEGRADATION SPECTRUM FOR 0.1% H_2O_2

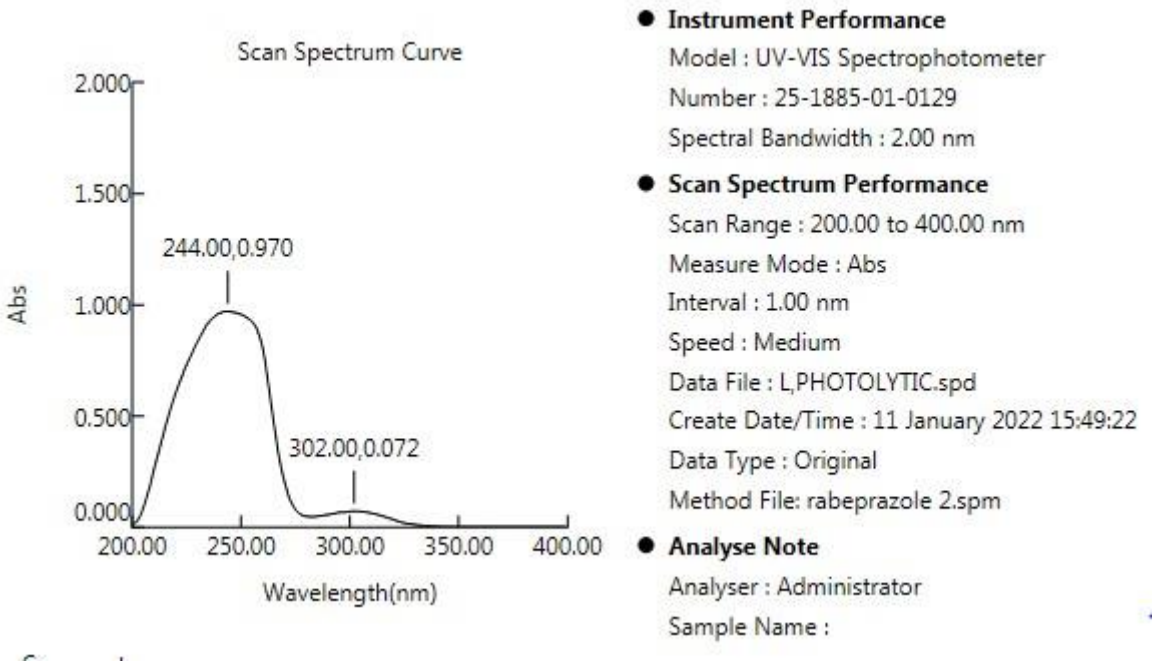

Fig. No. 5d: DEGRADATION SPECTRUM FOR PHOTOLYTIC

From the degradation data when levonorgestrel reacts with 0.1N HCl, the % degradation was found to be more than 20% hence we conclude that Levonorgestrel is unstable in 0.1N HCL, due to reduction. When reacting with 0.1N NaOH the % degradation was also found to be more than 20%, which was unstable due to the formation of salt. When levonorgestrel reacts with 0.1% H_2O_2, the % degradation was found to be more than 20%, so it was unstable due to

oxidation. While reacting with sunlight, levonorgestrel was found to be unstable because the % degradation was found to be more than 20%.

CONCLUSION

The proposed method was simple, sensitive, and reliable with good precision and accuracy. The method is specific while estimating the commercial formulation without the interference of excipients and the other additives. Hence it can be used for routine analysis of levonorgestrel in bulk and pharmaceutical formulations. The proposed method for stability study showed that there is appreciable degradation found in stress conditions of levonorgestrel. In addition to demonstrating specificity, a forced degradation study can be used to determine the degradation pathways and degradation product of the APIs that could form during storage and facilitate formulation, development, manufacturing, and packaging.

ACKNOWLEDGEMENT

We are thankful to Adhiparasakthi College of Pharmacy, Melmaruvathur for providing chemicals and laboratory facilities to carry out the work. We are highly grateful to Madras Pharmaceuticals Ltd, Chennai for providing Levonorgestrel.

ABBREVIATION

HPLC- High Performance Liquid Chromatography, RP-HPLC- Reverse Phase High Performance Liquid Chromatography, LC- Liquid Chromatography, MS- Mass Spectroscopy, UPLC- Ultra Performance Liquid Chromatograph, LOD- Limit of Detection, LOQ- Limit of Qantification.

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