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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATION OF LEVONORGESTREL IN BULK DOSAGE FORM

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Abstract:

A stability-indicating RP-HPLC method was developed and validated for the determination of Levonorgestrel in bulk dosage forms using Phenomenex C_{18} column (250 mm × 4.6 mm id, 5 µm particle size) as stationary phase; acetonitrile: water (80:20, v/v) as a mobile phase with flow rate of 1.0 ml/min. Quantification was achieved with Photo Diode Array detector at 241 nm. The retention time for Levonorgestrel was found to be 2.47 min. The linearity was obtained in the concentration range of 1-12 µg/ml for Levonorgestrel. Levonorgestrel was subjected to stress conditions including acidic, alkaline, oxidative, thermal, UV radiation and sunlight degradation conditions. Levonorgestrel was found more sensitive towards acidic, alkaline and oxidative degradations. The method was validated as per ICH guidelines.

Keywords: Levonorgestrel, RP-HPLC, Stability indicating, Validation, Recovery.

Introduction

Levonorgestrel (LNG) is (−)-13-ethyl-17-hydroxy-18, 19-dinor-17 alpha-pregn-4-en-20-yn-3-one. It is a synthetic progestin used as a progestin-only emergency contraceptive, and when administered at lower doses either alone or in combination with an estrogen, as an oral contraceptive. It is official in IP [1] and USP [2]. LNG has strong antiestrogenic effect that makes cervical mucus impenetrable to spermatozoa, thus preventing fertilization [3-4]. LNG has also been the progestogen of choice for inclusion in drug delivery systems such as implants, intrauterine devices, and intravaginal rings. Several analytical methods were described for determination of LNG in biological media or in pharmaceutical dosage forms including difference circular dichroism spectroscopy [5], membrane inlet mass spectrometry and desorption chemical ionization [6], first-derivative spectrophotometry [7], spectrophotometry with multivariate calibration technique [8], liquid chromatography-electron spray mass spectrometry [9-11], solid-phase extraction-liquid chromatography-diode array-mass spectrometry [12-14], solid-phase gas chromatography-mass spectrometry [15], capillary liquid chromatography and capillary elctrochromatography [16], micellar electrokinetic capillary chromatography [17] and HPLC with UV-detection [18].

MATERIALS & METHODS:

Apparatus:

The chromatography was performed on a Shimadzu (Japan) RP-HPLC instrument (LC-2010C_{HT}) equipped with PDA detector and LC-solution software, Phenomenex C_{18} column (250 mm × 4.6 mm id, 5µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad), a hot air oven (Grover, New Delhi, India) and an UV cabinet (CAMAG, Ancrom, Mumbai, India) were used in the study.

Reagents and materials:

LNG standard was kindly supplied as a gift sample from Famycare Pharmaceutical Ltd., Ahmedabad, Gujarat, India. The tablet formulation (Unwanted 72) containing 1.5 mg LNG was obtained from local market. Acetonitrile, Methanol, triple distilled water (S. D. Fine Chemicals Ltd., Mumbai, India) used were of HPLC grade. Hydrochloric Acid (AR grade, S. D. Fine Chemicals Ltd., Mumbai, India), Sodium Hydroxide (AR grade, S. D. Fine Chemicals Ltd., Mumbai, India), Hydrogen Peroxide (AR grade, S. D. Fine Chemicals Ltd., Mumbai, India), Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Chromatographic Conditions:

Stationary phase: Phenomenex C_{18} column (250 mm x 4.6 mm id, 5 µm particle size)
Mobile phase: Acetonitrile: Water (80: 20, v/v)
Flow rate: 1.0 ml/min
Injection volume: 20 µL
Temperature: 40 °C
Detection: At 241 nm using PDA detector.

Preparation of Solutions:

Preparation of standard stock solutions of LNG:

Accurately weighed LNG (10 mg) was transferred to a 100 ml volumetric flask and dissolved and diluted up to 100 ml methanol to obtain a standard stock solution (100 µg/ml).

Preparation of sample solution:

The average weight of 10 tablets was determined and was ground in a mortar. An accurately weighed amount of powder equivalent to 1.5 mg of LNG was transferred to 100 ml volumetric flask, dissolved in methanol and sonicated for 10 min. The content was filtered through Whatman filter paper and diluted up to mark with methanol. This solution contains 15 µg/ml of LNG. From the above solution, 4 ml was transferred to 10 ml volumetric flask and diluted up to mark with methanol to get the concentration of 6 µg/ml of LNG. An aliquot (20 µl) of sample solution was injected under the operating chromatographic condition as described above and responses were recorded.

Preparation of 1N HCl:

A solution of 1N HCl was prepared by taking 8.6 ml concentrated HCl in 100 ml volumetric flask and diluted up to mark with methanol.

Preparation of 0.5N NaOH:

A solution of 0.5N NaOH was prepared by dissolving 2 gm NaOH pallets in 100 ml methanol.

Preparation of 10% H_2O_2:

A solution of 10 % H_2O_2 was prepared by taking 10 ml of H_2O_2 in 100 ml volumetric flask and diluted up to mark with methanol.

Preparation of Calibration Curve:

Aliquots equivalent to 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml standard stock solutions of LNG were transferred into a series of seven 10 ml volumetric flasks separately and volume was adjusted up to the mark with methanol to get concentrations 1, 2, 4, 6, 8, 10 and 12 µg/ml of LNG. 20 µl of each of the solution were injected into HPLC system and analyzed. Calibration curve was obtained by plotting respective peak area against concentration in µg/ml and the regression equation was computed.

Method Validation:

The method was validated in compliance with ICH guidelines [19].

Linearity:

A stock solution of LNG (100 µg/ml) was prepared with methanol. From it various working standard solutions were prepared in the range from 1-12 µg/ml and injected in to system. The calibration plot (peak area of LNG vs. concentration of LNG) was generated by replicate analysis (n=5).

Method precision (Repeatability):

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of LNG (6 µg/ml without changing the parameters.

Intermediate Precision (Reproducibility):

Precision of the method was determined by performing interday variation and intraday variation in terms of %RSD. Intraday precision was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Interday precision was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by ICH guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

where, σ = the standard deviation of the response and
S = slope of the calibration curve

Accuracy (Recovery study):

To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%). A known amount of drug was added to pre-analyzed sample powder and percentage recoveries were calculated.

FORCE DEGRADATION STUDY:

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acidic, alkaline, oxidative, thermal, UV light, and sunlight degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

Acidic Degradation:

Acidic degradation was performed to force the degradation of LNG (10 µg/ml) to its primary degradation products by 1N HCl at 100 °C for 7 hrs.

Alkaline Degradation:

Alkaline degradation was performed to force the degradation of LNG (10 µg/ml) to its primary degradation products by 0.5N NaOH at 100 °C for 7 hrs.

Oxidative Degradation:

Oxidative degradation was performed to force the degradation of LNG (10 µg/ml) to its primary degradation products by 10% H_2O_2 at 100 °C for 7 hrs.

Thermal Degradation:

LNG powder was subjected at 100 °C in hot air oven for 7 days. After 7 days, solution having concentration of 10 µg/ml was prepared from subjected powder.

UV light degradation:

LNG powder was subjected to UV light in UV cabinet for 7 days. After 7 days, solution having concentration of 10 µg/ml was prepared from subjected powder.

Sunlight Degradation:

LNG powder was subjected to direct sunlight for 7 days. After 7 days, solution having concentration of 10 µg/ml was prepared from subjected powder.

RESULTS AND DISCUSSION:

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. Satisfactory results for LNG were obtained with a mobile phase comprising of acetonitrile: water (80: 20, v/v) with a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 241 nm based on peak area. The peak with clear baseline was obtained (Figure 2). The retention time for LNG was found to be 2.47 min (Figure 2). Linear correlation was obtained between peak areas of LNG vs. concentrations of LNG in the concentration ranges of 1-12 µg/ml (Figure 1). The mean recovery obtained was 99.09 ± 0.79 % for LNG which indicates accuracy of the proposed method. The proposed method was validated as per ICH guidelines. The %RSD value for LNG was found to be <2%, which indicates that the proposed method is repeatable. The low %RSD values of interday and intraday variations for LNG revealed that the proposed method is precise. LOD and LOQ values for LNG were found to be 0.29 µg/ml and 0.88 µg/ml, respectively. These data show that the proposed method is sensitive for the determination of LNG.

In the present investigation the LNG was subjected to its stability studies under different conditions as per the ICH guidelines. The result of acidic degradation shows degradation peak at 1.75 min along with the drug peak. The peak area shows that 31.52% degradation of the drug occurred when the drug was kept in 1N HCl at 100°C for 7 hours (Figure 5). The result of alkaline hydrolysis shows degradation peak at 1.56 min along with the drug peak. The peak area shows that 68.31% degradation of the drug occurred when the drug was kept in 0.5N NaOH at 100°C for 7 hours (Figure 6). The result of oxidative degradation shows degradation peak at 1.33 min along with the drug peak. The peak area shows that 43.14% degradation of the drug occurred when the drug was kept in 10% H_2O_2 at 100°C for 7 hours (Figure 7). The results of thermal, UV light and sunlight degradations were showing that there were no separate peak for degraded product and there were very low decrease in peak area of main drug. Thus it shows very minor degradation (Figure 8-10).

CONCLUSION:

A simple, sensitive, repeatable and specific stability indicating RP-HPLC method has been developed for the estimation of LNG. The method was validated for accuracy, precision, linearity, specificity, LOD & LOQ and robustness. In this proposed method the linearity is observed in the concentration range of 1-12 µg/ml for LNG at 241 nm. LNG was found to be more degraded when exposed to alkaline, oxidative and acidic degradation conditions and very less degraded when exposed to thermal, UV light and sunlight degradation conditions. The method can be used for the routine analysis of LNG in pharmaceutical dosage form without any interference of excipients.

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ABBREVIATIONS:

LNG: Levonorgestrel
ICH: International Conference on Harmonization
IP: Indian Pharmacopoeia
USP: United States Pharmacopoeia
UV: Ultraviolet
RP-HPLC: Reverse Phase High Performance Liquid Chromatography
HPLC: High Performance Liquid Chromatography
PDA: Photo Diode Array
RSD: Relative Standard Deviation
SD: Standard Deviation
LOD: Limit of Detection
LOQ: Limit of Quantification
HCl: Hydrochloric Acid
NaOH: Sodium Hydroxide
H_2O_2: Hydrogen Peroxide

Table 1. Regression analysis data and summary of validation parameter for the proposed method

Parameters	Data
Concentration range (µg/ml)	1-12
Slope	76610.43
Intercept	5729.80
Correlation coefficient	0.9990
LOD	0.29
LOQ	0.88
Accuracy	99.09 ± 0.79%
Repeatability (%RSD, n=6)	1.21
Precision (%RSD) **Interday (n=3)** **Intraday (n=3)**	 0.20 - 1.34 0.19 - 1.30
% Assay	98.33 ± 0.69

Table 2. Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± %RSD (n=3)
LNG	I	6	50 %	99.45 ± 1.07
	II	6	100 %	99.15 ± 0.76
	III	6	150 %	98.67 ± 0.54

Table 3. System suitability test parameters of LNG for the proposed method

Parameters	LNG ± %RSD (n=6)
Retention Time (minutes)	2.45 ± 0.94
Tailing Factor	0.90 ± 0.51
Theoretical Plate	2095 ± 1.99
Resolution	2.05 ± 0.89

Table 4. Analysis of tablet formulation of LNG by proposed method (n=6)

Formulation	Label Claim (mg)	Amount Found (mg)	% Label claim ± %RSD (n=3)
LNG	1.5	1.47	98.33 ± 0.69

Table 5. Results of force degradation studies of LNG

Sr. No.	Stress condition	Impurity formed	Retention Time (min)	% Degradation
1	Acidic – 1N HCl	Impurity A	1.75 ± 0.20	-
		LNG	2.45 ± 0.68	31.52
2	Alkaline – 0.5N NaOH	Impurity B	1.56 ± 1.71	-
		LNG	2.47 ± 0.95	68.31
3	Oxidative – 10% H_2O_2	Impurity C	1.33 ± 0.43	-
		LNG	2.45 ± 1.24	43.14
4	Thermal	-	-	-
		LNG	2.47 ± 1.57	5.08
5	UV light	-	-	-
		LNG	2.45 ± 0.21	3.89
6	Sunlight	-	-	-
		LNG	2.47 ± .067	4.59

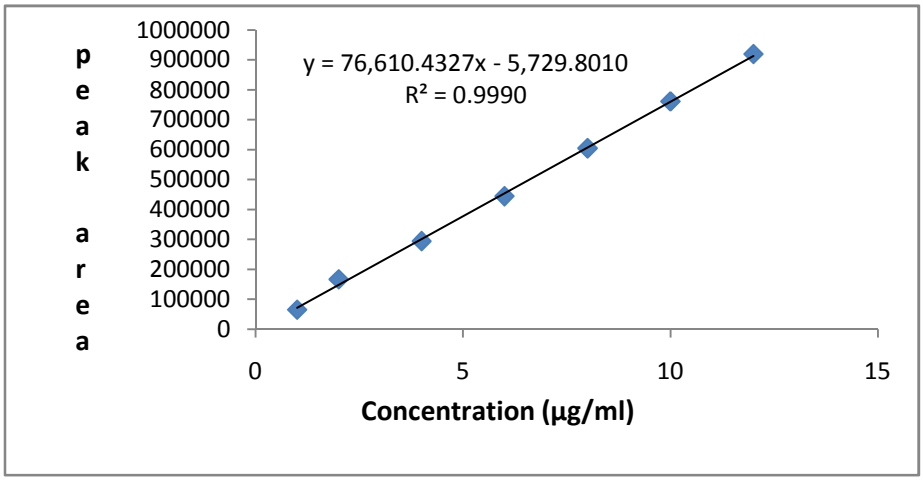

Figure 1. Calibration curve of LNG at 241 nm

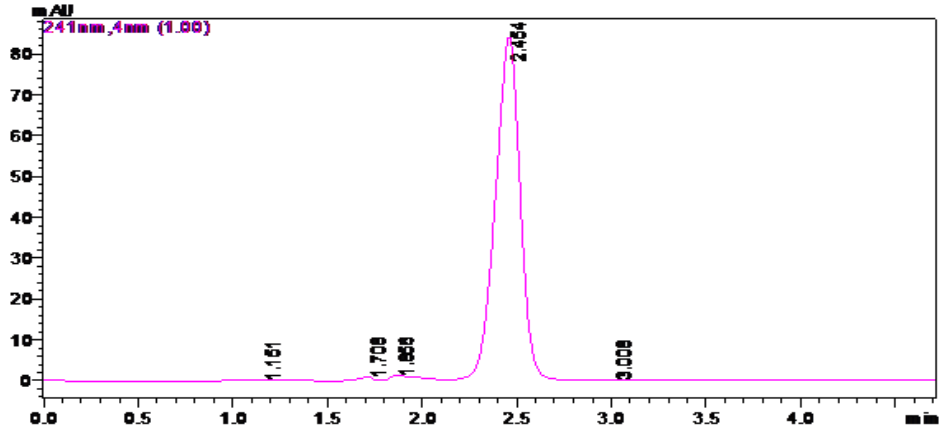

Figure 2. Chromatogram of standard LNG (10 µg/ml) at 241 nm

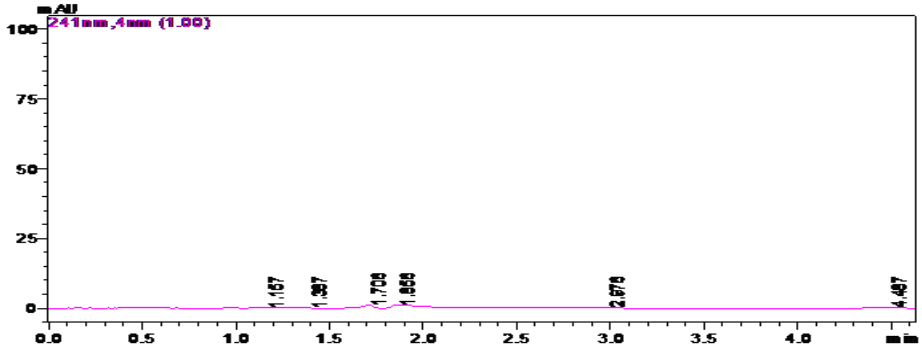

Figure 3. Chromatogram of blank solution at 241 nm

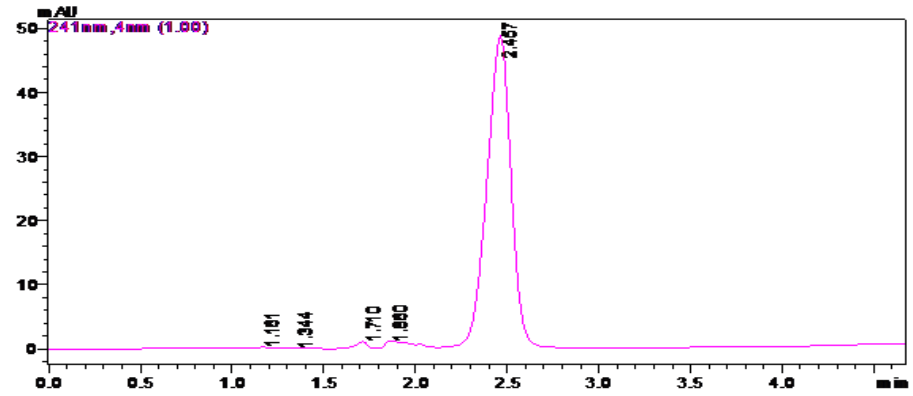

Figure 4. Chromatogram of sample LNG (6 µg/ml) at 241 nm

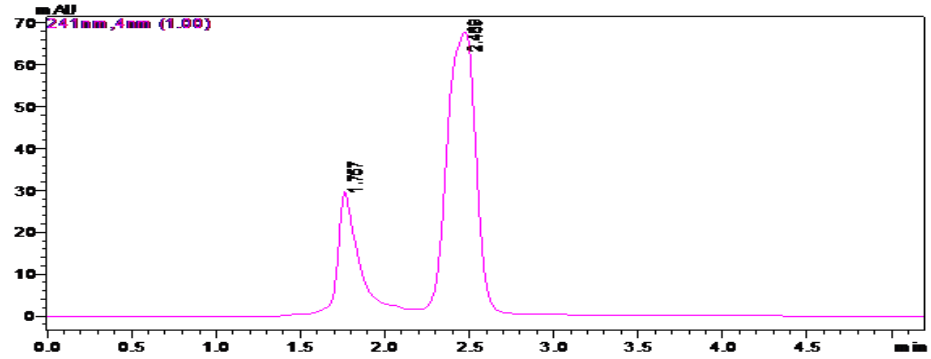

Figure 5. Chromatogram of LNG (10 µg/ml) at 241 nm in 1N HCl

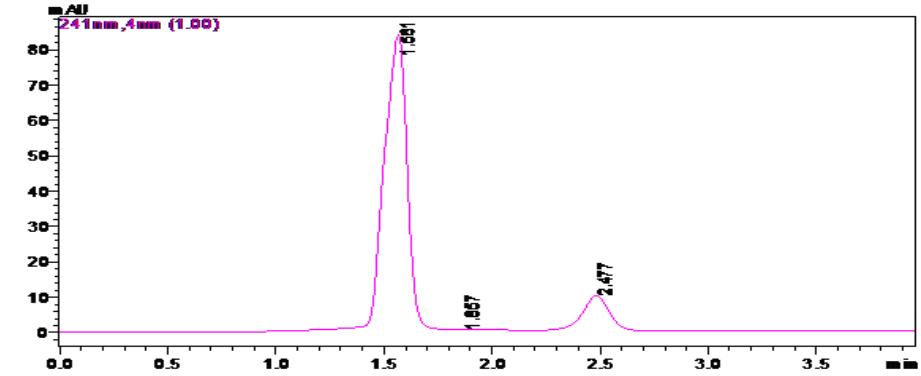

Figure 6. Chromatogram of LNG (10 µg/ml) at 241 nm in 0.5N NaOH

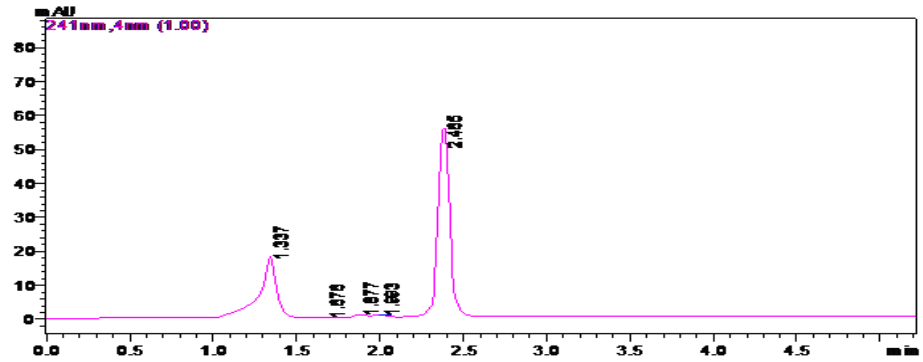

Figure 7. Chromatogram of LNG (10 µg/ml) at 241 nm in 10% H_2O_2

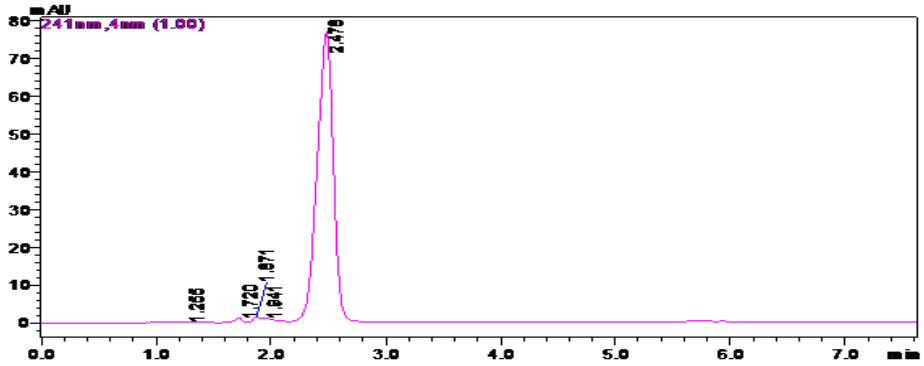

Figure 8. Chromatogram of LNG (10 µg/ml) at 241 nm in Thermal Conditions

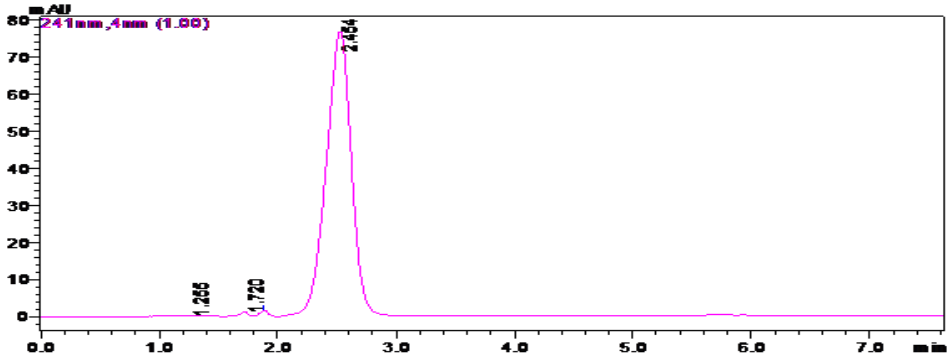

Figure 9. Chromatogram of LNG (10 µg/ml) at 241 nm in UV Light

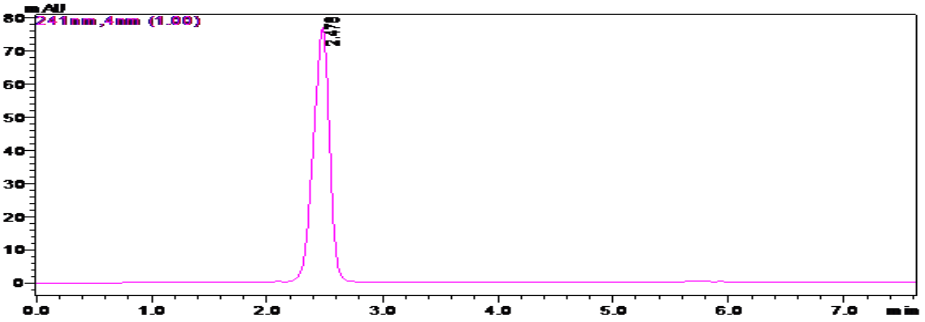

Figure 10. Chromatogram of LNG (10 µg/ml) at 241 nm in Sunlight

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