

# Development and Validation of Novel Analytical Simultaneous Estimation Based UV Spectrophotometric Method for Doxycycline and Levofloxacin Determination

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**Abstract:** The thorough literature study uncovered that none of the most perceived pharmacopeias or any journals includes a method for simultaneous estimation of Doxycycline and Levofloxacin in combination by UV/Visible spectroscopy. So, it was felt fundamental to build up a system that will serve as a solid, precise UV technique for the simultaneous estimation of Doxycycline and Levofloxacin. DOXH and LVXH showed  $\lambda_{max}$  at 273nm and 287nm respectively, and iso-absorptive point at 280nm in Phosphate buffer pH 6.8 prepared in Water: Methanol (80:20) dissolvable solvent system. Beer Lambert's law obeyed by both drugs within the concentration range of 2-20  $\mu\text{g/ml}$  &  $r^2$  values of 0.9999 and 0.9998, which shows the good linearity. The method has been validated statistically and quantitatively regarding linearity, precision, LOD, LOQ, accuracy, and specificity according to the ICH guidelines. LOD for DOXH and LVXH were found to be 1.41 and 0.63  $\mu\text{g/ml}$ , the LOQ was 4.30 and 1.92  $\mu\text{g/ml}$ , respectively. Percent recovery at recovery level of 80%, 100% & 120% for DOXH was found to be 99.7, 99.66 & 99.69 & for LVXH 99.58, 99.66 & 99.63 respectively. Intra-day, Inter-day & precision analysis by different analyst was found to be 0.767, 0.563, 0.440 %RSD for DOXH & 0.507, 0.532, 0.708 % RSD for LVXH. Sandell's sensitivity was discovered to be adequate, and this shows that extremely less measure of the two medications can be successfully recognized by this technique. Finally, it was concluded, the developed & validated method was helpful and appropriate for regular quality analysis and simultaneous determination of drug products containing DOXH and LVXH in combination.

**Keywords:** doxycycline hyclate; levofloxacin hemihydrate; synthetic mixture; simultaneous estimation; UV- Visible spectrophotometric method.

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## 1. Introduction

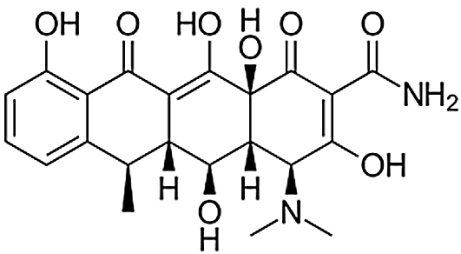
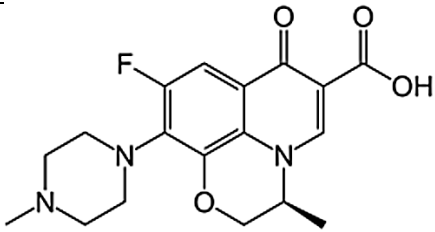
Doxycycline hyclate (DOXH),  $\text{C}_{24}\text{H}_{33}\text{ClN}_2\text{O}_{10}$  (CAS No. 24390-14-5), is a hygroscopic, yellowish powder, freely soluble in water & soluble in methanol[1] having Mol. Wt. 512.94 g/mol[2]. It is a wide range of effective semisynthetic[3] long-acting hydrochloride hemiethanol hemihydrate of Doxycycline got from Oxytetracycline[4, 5] having considerably more dissolvability power than doxycycline monohydrate, because of which it is broadly used in drug production[6]. It shows broad-spectrum effectiveness towards gram +ve and gram -ve microorganisms, including *Bartonella*, *Streptococcus Pyogenes*, *Hemoplasma*, *Spirochetes*, *Chlamydia Elis*, *Enterococci*, *Ehrlichia*, *Actinomyces sp.*, *Anaplasma Nocardia*, *Toxoplasma*, <https://biointerfaceresearch.com/>

*Plasmodium Species*, *Mycoplasma*, *Protozoa*, and a few anaerobic varieties[7]. It inhibits matrix metalloproteinases (MMP's)[8] which are engaged with various immune and inflammatory reactions, for example, periodontitis, gum disease, degenerative rheumatic illnesses, and degenerative vascular issues[9, 10] consequently it is utilized to cure periodontitis and related risk problems like respiratory issues[11], diabetes mellitus[12], cardiovascular problems[13], foetopahtic changes in incubation period[14], alveolar bone misfortune[15] and so on. DOXH is acted by inhibiting protein synthesis and the alteration of cytoplasmic film penetrability inside the susceptible organism[5]. DOXH is additionally used in the cure and treatment of relapsing fever, acne, chronic prostatitis, respiratory infections, sinusitis, Lyme sickness, syphilis, exacerbations of bronchitis in patients with COPD, brucellosis, chronic intestinal amoebiasis, non-gonococcal urethritis, cervicitis, urinary tract infection, chlamydia, provocative pelvic illness, rickettsial diseases, and threatening radiations that are regularly connected with malignancies[16] and ovary, lung & breast cancer, exact treatment (for beginning therapy of all blended disease) and other sexually transmitted diseases[4, 5, 17]. Indian Pharmacopeia[18], British Pharmacopeia[19], United States Pharmacopeia[20], European Pharmacopeia[21], and Japanese Pharmacopeia[22] having DOXH official monograph.

Levofloxacin hemihydrate (LVXH),  $C_{36}H_{42}F_2N_6O_9$  (CAS No. 138199-71-0), is an off-white to yellow, odorless translucent powder, sparingly soluble in water & methanol, having Mol. wt. 740.7 g/mol.[23] LVXH is an L-isomer of ofloxacin[24], second-generation synthetic fluoroquinolones alluded to as respiratory quinolone[25]. It shows broad-spectrum bactericidal effectiveness towards gram +ve and gram -ve aerobes[26, 27] shows more prominent movement towards gram-positive microbes yet lesser action toward gram-negative microorganisms. It hinders the supercoiling movement of bacterial DNA gyrase and topoisomerase – II and IV, fundamental proteins in the multiplication of bacterial DNA, which brings about the stopping of DNA replication. Topoisomerase IV is necessary to separate DNA that has been imitated preceding bacterial cell division[24]. Bacteria are unable to divide due to an interrupted cell division cycle as DNA is not separated. As supercoiling of DNA is caused by DNA gyrase, it will fit in the recently shaped cells[28]. The said mechanisms are responsible for the killing of the bacteria. LVXH has application in the treatment of airway infections[29], conjunctivitis, urinary tract infections, acute bacterial sinusitis, chronic bronchitis, chronic prostatitis, mastitis, community-acquired and nosocomial pneumonia, abdominal infections, gastroenteritis, topical infections, and acute pyelonephritis[30–32]. It is a principal drug in the management of MDR tuberculosis[33]. It is likewise used to treat irresistible loose bowels brought about by *E. coli*, *Campylobacter jejuni*, and *Shigella* microorganisms. It is official in the United States pharmacopeia[34]. Chemical Structures and IUPAC names of Doxycycline hyclate and Levofloxacin hemihydrate were given in Table 1. The literature survey uncovers a few logical techniques for Doxycycline estimation viz. UV method[35, 36], Derived spectroscopic methods[37], UV spectrophotometric absorption ratio method[38], Chemiluminescence[39], HPLC[40–42], HPLC-UV[43], Thin-layer chromatography[44], HPTLC[45], RP-HPLC[46–48], Optical fiber sensor[49], Sequential injection chromatographic analysis[50], Lanthanide sensitized luminescence[51], IR spectroscopy[52], Solid surface phosphorescence[53], Flow-injection analysis/merging zones technique[54], HPLC-DAD and LC-ESI-MS analysis[3], Internal solid contact sensor based on conducting polypyrrole[55], HPLC-UV and LC-MS-MS[56], HPLC-MS[57], Micellar electrokinetic

capillary chromatography[58], Ion-selective electrode potentiometry[59], Chemometric-Assisted Spectrophotometric Method[60], Capillary electrophoresis[61], Fluorimetry[62], TLC-fluorescence scanning densitometry[63], Titrimetry[64], Cyclodextrin based fluorosensor[65] and techniques for Levofloxacin estimation viz. UV method[66–68], HPLC[69], RP-HPLC[70–73], HPTLC[74], HPLC–FLD–DAD[75], HPLC-MS[76], Micellar liquid chromatography[77, 78], Spectrofluorometric methods[79, 80], UPLC[81], UPLC-MS/MS[82], HPLC with UV detection[83, 84], Vibrational Spectroscopy[85], Fluorometric detection[86], Atomic Absorption Spectrometry[87], HPLC - Tandem mass spectrometry[88], RP-UFLC[89], Capillary electrophoresis with electrochemiluminescence detection[90], Adsorptive square-wave anodic – stripping voltammetry[91], Chemiluminescence[92], Hydrotropy and first derivative UV spectroscopy[93], Conductometric, Potentiometric[94] and Flow injection analysis method[95], which are monotonous, awkward, slow processing time, expensive process due to need of costly reagents, use high degree temperatures and some buffer solutions, require extraction, have been accounted for quantitative assessment of DOXH and LVXH alone and in combine dosage forms. The standard procedure is given in the British Pharmacopoeia[96].

**Table 1.** Structure and IUPAC Name of Doxycycline hyclate[97] and Levofloxacin hemihydrate[98].

Drug Structure	IUPAC Name
 <p>Doxycycline</p>	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>R</i> ,12 <i>aR</i> )-4-(dimethylamino)-1,5,10,11,12 <i>a</i> -pentahydroxy-6-methyl-3,12-dioxo-4 <i>a</i> ,5,5 <i>a</i> ,6-tetrahydro-4 <i>H</i> -tetracene-2-carboxamide;ethanol;hydrate
 <p>Levofloxacin</p>	(2 <i>S</i> )-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0]trideca-5(13),6,8,11-tetraene-11-carboxylic acid

All of these strategies are less delicate, including tedious systems like warming and extraction, exorbitant reagents, etc. Also, reported techniques were not much cost-effective as far as the above factors, so the current investigation was embraced. The thorough literature study uncovered that none of the most perceived pharmacopoeias or any journals includes simultaneous determination technique for DOXH and LVXH in combination by UV/Visible spectroscopy. Obvious spectrophotometry, due to straightforwardness, cost-effectivity, selectivity, reasonable exactness, and accuracy, have stayed serious in a period of chromatographic methods for drug examination. So, it was felt fundamental to build up a system that will serve as a solid, precise UV technique for the simultaneous estimation of DOXH and LVXH. The current examination was completed in the perspective of setting up a simple, quick, precise, cost-effective, and robust UV technique for simultaneous determination of DOXH and LVXH in combination drug products.

The main objective of the current study is to develop and validate the UV-Visible spectrophotometric techniques for the quantitative assessment of DOXH and LVXH in a synthetic mixture which is also applicable for their combined pharmaceutical formulations to build up exactness and precision of determining results without using expensive reagents and instrumentation.

## 2. Materials and Methods

Analytically pure free samples of DOXH and LVXH were procured from Micro Labs Limited, Bangalore and The Madras Pharmaceuticals, Karappakam, Chennai, respectively. Double distilled water, methanol, and the other reagents used were of AR grade.

### 2.1. Instruments.

A double beam UV-Visible spectrophotometer (Shimadzu UV-1800), 10mm matched quartered cell with UV-probe software, pH meter, Ultra sonicator, and calibrated Precision balance model Citizen Cy220 (0.1mg sensitivity) were used for the experimental purpose.

### 2.2. Solvent system.

After several trial-and-error experimentations on various solvents in different proportions, finally Distilled water and Methanol mixture in the ratio 80:20 was selected as a solvent system for method development procedure considering solubility of both the drugs, i.e., DOXH and LVXH in the respective solvent.

### 2.3. Preparation of Phosphate Buffer pH 6.8.

Dissolve 28.80gm of Disodium Hydrogen Phosphate and 11.45gm of Potassium Dihydrogen Phosphate in the required quantity of selected solvent system to produce 1000 ml.[99]

### 2.4. Standard stock solution of DOXH.

A precisely weighed amount of DOXH (10 mg) was taken in a 10ml volumetric flask, dissolved, and diluted with prepared Phosphate Buffer pH 6.8 to acquire a working-standard solution of 1000 $\mu$ g/ml concentration. From this solution adequate amount was pipetted out and diluted with the prepared Phosphate Buffer pH 6.8 to acquire working dilutions in a concentration range of 2 to 20 $\mu$ g/ml.

### 2.5. Standard stock solution of LVXH.

A precisely weighed amount of LVXH (10 mg) was taken in a 10ml volumetric flask, dissolved, and diluted with prepared Phosphate Buffer pH 6.8 to acquire a working-standard solution of 1000 $\mu$ g/ml concentration. From this solution adequate amount was pipetted out and diluted with the prepared Phosphate Buffer pH 6.8 to acquire working dilutions in a concentration range of 2 to 20 $\mu$ g/ml.

2.6. *Mixed standard stock solution.*

The mixed standard stock solution of DOXH and LVXH in the ratio of 1:1 was prepared from standard stock solutions (1000µg/ml) using Phosphate Buffer pH 6.8.

2.7. *Absorption maxima and Iso-absorptive point determination.*

The standard solution (10µg/ml) was examined in the range mode over the scope of 200-400 nm utilizing a 100nm/min scanned speed, 1 cm quartz cell for absorption maxima determination, and overlain spectra preparation.

2.8. *Calibration curve preparation.*

Working dilutions of 2 - 20 µg/ml concentrations of DOXH and LVXH were prepared using Phosphate Buffer pH 6.8. Absorbance was recorded for DOXH and LVXH at 273nm and 287nm, respectively, and a standard calibration curve was prepared.

2.9. *Absorbance additivity study.*

Absorbance additivity data was obtained by measuring the absorbance of working standard dilutions of individual drugs (2µg/ml) and their mixture (1:1) at selected wavelengths, i.e., 273nm and 287nm.

2.10. *Absorptivity determination.*

The absorbances were estimated at the chosen wavelengths, i.e., 273nm and 287nm that are absorption maxima's of DOXH and LVXH individually and absorptivity (A 1%, 1 cm) of DOXH and LVXH at  $\lambda_{max}$ , i.e., 273nm and 287nm, respectively, were calculated from the mean of three different absorbance readings by using the following formula.

$$A (1\%, 1 \text{ cm}) = \text{Absorbance/Concentration (g/100ml)}$$

Finally, the mean of all absorptivity values was calculated as  $a_x$  and  $a_y$  at 273nm and 287nm, respectively.

2.11. *Simultaneous equation method development.*

On the off chance that a mixture has two different absorbing drugs, which shows absorbance at the  $\lambda_{max}$  of the each other, it very well might be feasible to estimate the two drugs by the procedure of simultaneous equation. Two wavelengths chosen for the technique are  $\lambda_1$  - 273nm and  $\lambda_2$  - 287nm that is absorption maxima's of DOXH and LVXH individually in Phosphate Buffer pH 6.8. Concentrations in the given mixture were estimated by using the following equations-

By considering DOXH and LVXH having concentrations,  $C_x$  and  $C_y$  respectively, in a synthetic mixture, the following two equations were designed at  $\lambda_1$  ( $\lambda_{max} - 273\text{nm}$ ) and  $\lambda_2$  ( $\lambda_{max} - 287\text{nm}$ ) as:

$$\text{At } \lambda_1 (\lambda_{max} - 273\text{nm}), A_1 = a_{x1} b C_x + a_{y1} b C_y \dots \dots \dots \text{Equ. 1}$$

$$\text{At } \lambda_2 (\lambda_{max} - 287\text{nm}), A_2 = a_{x2} b C_x + a_{y2} b C_y \dots \dots \dots \text{Equ. 2}$$

where,

$C_x$  &  $C_y$  = DOXH and LVXH Concentrations respectively.

$A_1$  &  $A_2$  = Absorbance of DOXH and LVXH synthetic mixture at 273 nm & 287 nm respectively.

$a_{x1}$  &  $a_{x2}$  = DOXH Absorptivity value at 273 nm & 287 nm respectively.

$a_{y1}$  &  $a_{y2}$  = LVXH Absorptivity value at 273 nm & 287 nm respectively.

$b = 1$ , for 1 cm cell measurement

Rearranging Equ. 2

$$C_y = A_2 - a_{x2} C_x / a_{y2} \dots\dots\dots \text{Equ. 3}$$

Substitute this value of  $C_y$  in Equ. 1 & after rearranging we get:

$$C_x = A_1 a_{y2} - A_2 a_{y1} / a_{y2} a_{x1} - a_{x2} a_{y1} \dots\dots\dots \text{Equ. 4}$$

$$C_y = A_1 a_{x2} - A_2 a_{x1} / a_{x2} a_{y1} - a_{x1} a_{y2} \dots\dots\dots \text{Equ. 5}$$

### 2.12. Validation protocol.

ICH Q2B guidelines were followed for the Linearity, Range, Precision, Specificity, LOD, LOQ, Sensitivity, and Accuracy studies which were performed for the validation of the proposed method. [100, 101].

#### 2.12.1. Linearity and range.

Various effective concentrations of DOXH and LVXH standard solution were investigated for assessing the linearity & range[102].

#### 2.12.2. Precision.

The closeness of agreement (level of dispersing) between a series of determinations obtained from multiple sampling of the similar homogeneous sample under the endorsed conditions is known as precision. Precision was completed by performing inter-day, intraday, and different analysts on the same day, utilizing 10µg/ml concentration. In an intraday variation, the absorbances were estimated multiple times in a day. In Inter-day variation, the sample was analyzed on three successive days. %RSD values were determined, which ought to be under 2 % [103, 104].

#### 2.12.3. Specificity.

Spectra of both the standard solution and the prepared drug sample solutions for the study was recorded to affirm the specificity of the proposed analytical method [105, 106].

#### 2.12.4. Limit of detection.

The minimum concentration of a sample that can be estimated but not necessarily as an exact value is known as the Limit of detection (LOD). It can be obtained by determining the signal-to-noise ratio, using International Conference on Harmonization (ICH) guidelines Q2 (R1) formula as given below:

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

where,  $\sigma$  = Standard deviation of the y-intercept of regression lines,

$S$  = Slope of a calibration curve

#### 2.12.5. Limit of Quantitation.

The minimum concentration of a sample that can be quantitatively estimated with suitable precision and accuracy is known as the Limit of quantification (LOQ).

$$\text{LOQ} = 10 \times \sigma / s$$

where,  $\sigma$  = Standard deviation of the y-intercept of regression lines,

S = Slope of a calibration curve

#### 2.12.6. Sensitivity.

Sensitivity refers to the smallest amount that can be precisely estimated. It additionally shows the limit of the technique to record or gauge small variations in concentrations. On account of spectrophotometric strategies, a parameter known as "Sandell's Sensitivity" is utilized to assess the method's sensitivity. It is the amount needed to give an absorbance of 0.001 units in one square centimeter way.

#### 2.12.7. Accuracy.

The accuracy determination was performed using the standard addition method. The pre-quantified 10 $\mu$ g/ml sample solution of DOXH and LVXH were spiked with an extra 80, 100, and 120% of the standard DOXH and LVXH pure drug. Absorbances were measured at 273nm, and 287 nm ( $\lambda_{\text{max}}$  of DOXH and LVXH, respectively), and the concentration of both drugs can be determined. These mixtures were analyzed, and %RSD values were estimated by the proposed developed method. The procedure was repeated thrice. The percentage recovery of the samples, %RSD, and the percentage were calculated at each concentration level[104, 107].

### 3. Results and Discussion

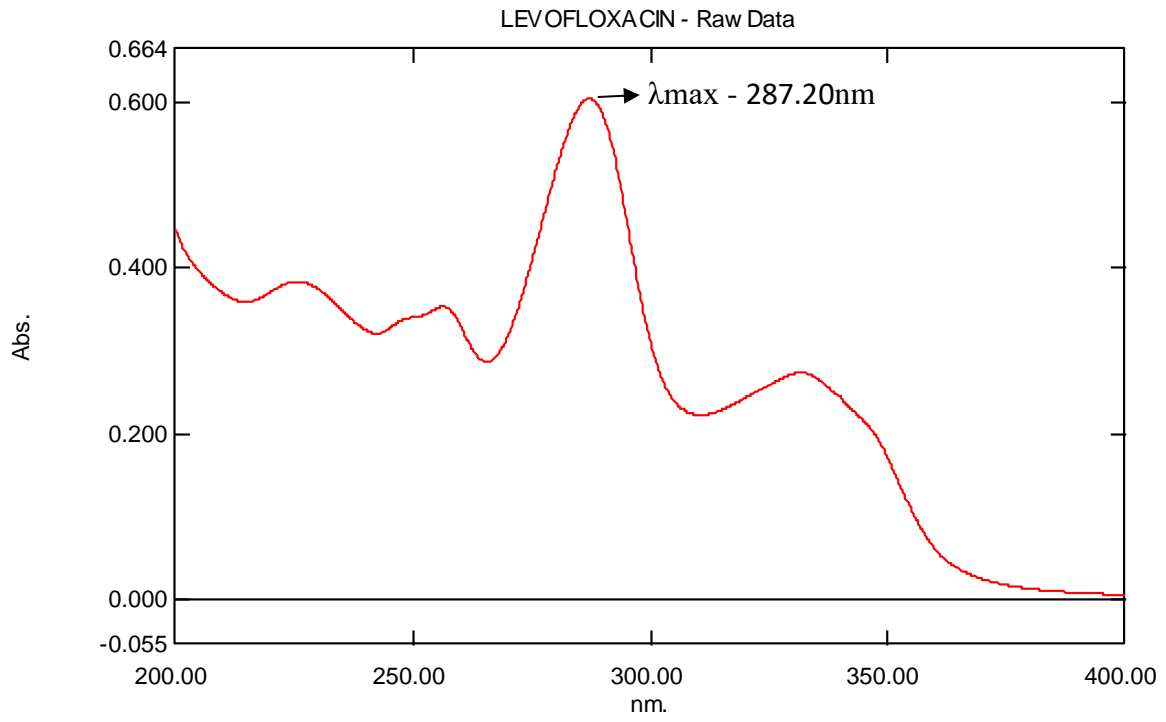
The proposed developed novel analytical method was found to be sensitive, simple, economical, accurate, and precise. The given method has been validated according to ICH guidelines.

#### 3.1. Absorption maxima and Iso-absorptive point determination.

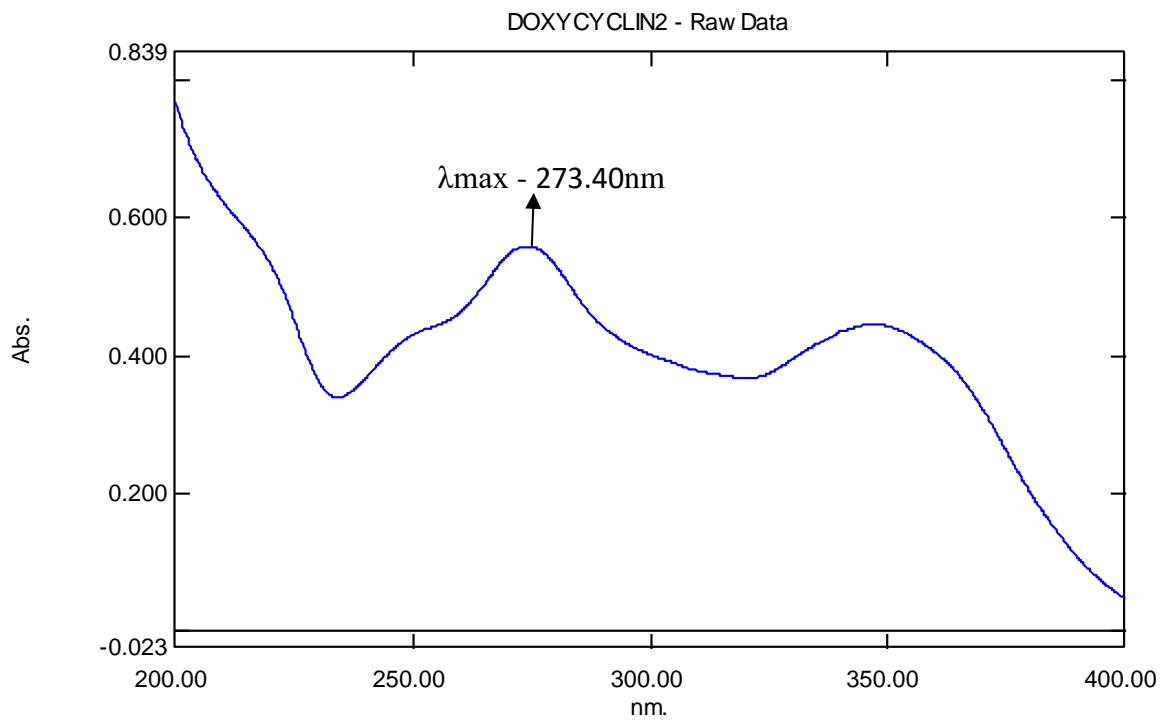
DOXH and LVXH showed an absorbance maxima peak at  $\lambda_{\text{max}}$  273nm and 287nm separately and were selected to develop the simultaneous equation method. Overlain spectra of the DOXH and LVXH depicted the occurrence of two peaks at 273nm and 287nm. Additionally, an iso-absorptive point was observed at 280nm (Figure 1).

#### 3.2. Calibration curve preparation.

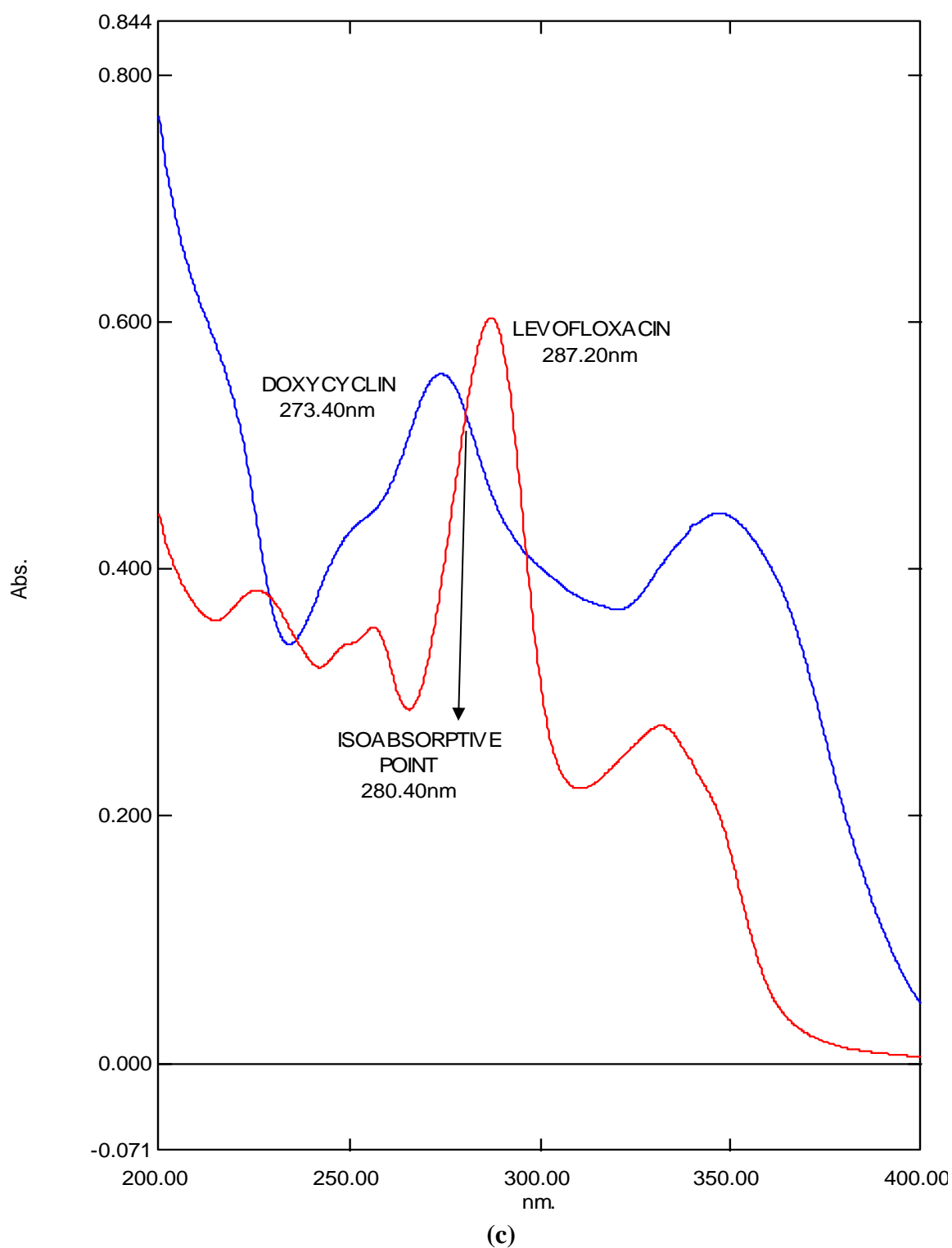
The absorbance of DOXH is obtained in a range of 0.155 to 1.073 at 273nm, and for LVXH is obtained in a range of 0.123 to 1.220 at 287nm. A standard calibration curve was obtained linear with  $r^2$  estimation of 0.9999 and 0.9998. (Table 2 ) (Figure 2)



(a)



(b)



**Figure 1.** (a) Spectra of Doxycycline hyclate ( $\lambda$  max- 273nm); (b) Levofloxacin hemihydrate ( $\lambda$  max- 287nm); (c) Overlain Spectra of Doxycycline hyclate ( $\lambda$  max- 273nm) and Levofloxacin hemihydrate ( $\lambda$  max- 287nm) showing the iso-absorptive point at 280 nm.

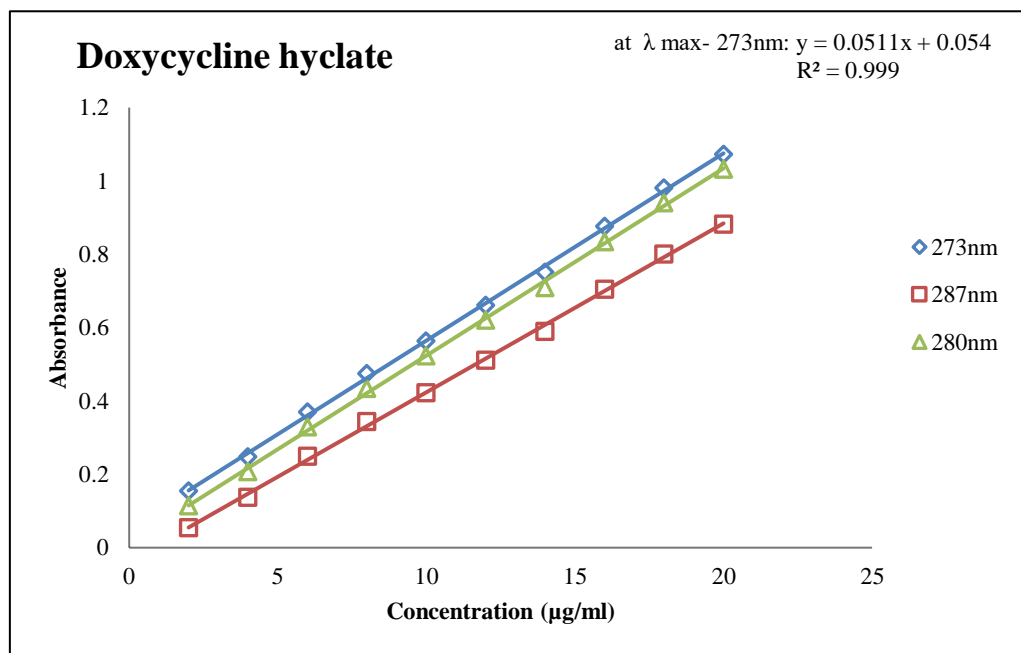
**Table 2.** Calibration curve absorbance readings and Absorptivity of DOXH and LVXH.

Sr. No.	Conc. ( $\mu\text{g/ml}$ )	Doxycycline hyclate				Levofloxacin hemihydrate			
		Absorbance		Absorptivity		Absorbance		Absorptivity	
		273nm	287nm	273nm	287nm	273nm	287nm	273nm	287nm
1	2	0.155	0.054	775	270	0.031	0.123	155	615
2	4	0.248	0.137	620	342.5	0.072	0.249	180	622.5
3	6	0.371	0.250	618.3	416.6	0.174	0.368	290	613.3
4	8	0.475	0.344	593.7	430	0.255	0.494	318.7	617.5

Sr. No.	Conc. (µg/ml)	Doxycycline hyclate				Levofloxacin hemihydrate			
		Absorbance		Absorptivity		Absorbance		Absorptivity	
		273nm	287nm	273nm	287nm	273nm	287nm	273nm	287nm
5	10	0.564	0.423	564	423	0.376	0.608	376	608
6	12	0.662	0.511	551.6	425.8	0.456	0.727	380	605.8
7	14	0.751	0.590	536.4	421.4	0.524	0.856	374.2	611.4
8	16	0.876	0.705	547.5	440.6	0.603	0.962	376.8	601.2
9	18	0.982	0.801	545.5	445	0.694	1.089	385.5	605
10	20	1.073	0.882	536.5	441	0.781	1.220	390.5	610
Mean (ax)				588.85	405.59	Mean (ay)		322.67	610.97

**Table 3.** Absorbance readings of Doxycycline hyclate:Levofloxacin hemihydrate (1:1) synthetic mixture.

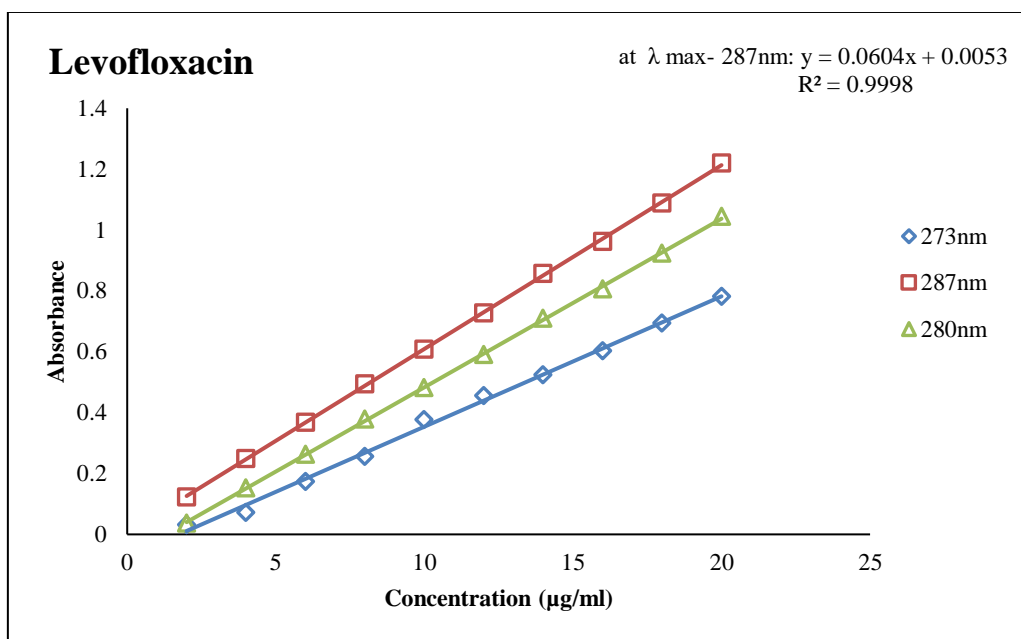
Sr. No.	Conc. (µg/ml)	Absorbance		
		273nm	287nm	280nm
1.	2:02	0.083	0.0785	0.0655
2.	4:04	0.14	0.173	0.16
3.	6:06	0.2425	0.279	0.266
4.	8:08	0.325	0.379	0.366
5.	10:10	0.42	0.4655	0.4525
6.	12:12	0.499	0.559	0.546
7.	14:14	0.5675	0.653	0.64
8.	16:16	0.6595	0.7535	0.7405
9.	18:18	0.748	0.855	0.842
10.	20:20	0.827	0.951	0.938



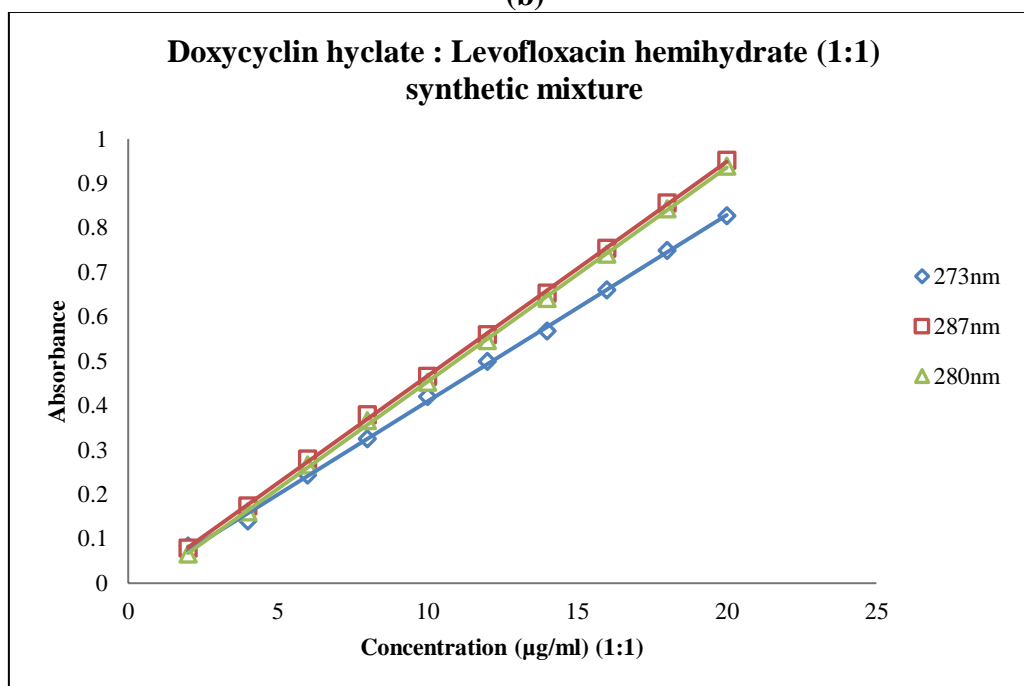
(a)

### 3.3. Absorbance additivity study.

This study shows the significant difference between the theoretical and practical absorbance of a synthetic mixture (Table 4).



(b)



(c)

**Figure 2.** Calibration Curve of (a) Doxycycline hyclate; (b) Levofloxacin hemihydrate; (c) Doxycyclin hyclate:Levofloxacin hemihydrate (1:1) synthetic mixture at 273 nm, 287 nm & 280 nm.

**Table 4.** Absorbance additivity study.

Sr. No.	Absorbance				Theoretical Absorbance of Mixture		Practical Absorbance of Mixture	
	DOXH		LVXH		273nm	287nm	273nm	287nm
	273nm	287nm	273nm	287nm				
1.	0.155	0.054	0.031	0.123	0.186	0.177	0.184	0.174
2.	0.156	0.052	0.033	0.124	0.189	0.176	0.187	0.175
3.	0.156	0.053	0.031	0.121	0.187	0.174	0.186	0.172
4.	0.153	0.054	0.03	0.123	0.183	0.177	0.181	0.174
5.	0.155	0.056	0.032	0.121	0.187	0.177	0.185	0.173

3.4. Absorptivity determination.

Absorptivity readings of DOXH and LVXH at 273nm and 287nm are shown in Table 2. Average absorptivity values of DOXH were found to be 588.85 at 273nm and 405.59 at 287nm. Average absorptivity values of LVXH were found to be 322.67 at 273nm and 610.97 at 287nm.

3.5. Simultaneous equation method development.

To find out the concentration of DOXH and LVXH in a synthetic mixture, the following simultaneous equations were used to calculate the concentration of individual drugs:

For DOXH concentration measurement,

$$C_x = \frac{A_1 a_{y2} - A_2 a_{y1}}{a_{y2} a_{x1} - a_{x2} a_{y1}}$$

For LVXH concentration measurement, use Equ. 3 i.e.

$$C_y = \frac{A_2 - a_{x2} C_x}{a_{y2}}$$

This equation is further used and validated for a synthetic mixture's DOXH (Cx) and LVXH (Cy).

3.6. Validation protocol.

3.6.1. Linearity and range.

Selected methods showed linearity within the 2-20 µg/ml concentration range for DOXH and LVXH, and the correlation coefficient values, r<sup>2</sup> for UV spectroscopy, were 0.9999 and 0.9998 at λ<sub>max</sub> 273nm and 287nm, respectively. Absorbance vs. concentration relation of DOXH and LVXH shows linear correlation. The linearity of the connection among absorbance and concentration was dictated by plotting the calibration curve for DOXH and LVXH separately shown in Figure 2 (a) & (b), respectively. The results of the linearity are given in Table 2.

3.6.2. Precision.

Intra-day precision of the proposed method was found to be 0.767 % RSD for DOXH and 0.507 % RSD for LVXH, Inter-days precision of the proposed method was found to be 0.563 % RSD for DOXH, and 0.532 % RSD for LVXH, Precision analysis by the different analyst of the method was found to be 0.440 % RSD for DOXH and 0.708 % RSD for LVXH (Table 5).

**Table 5.** Precision analysis by Intraday, Interday, and Different Analyst parameters.

Sr. No.	Intraday		Interday		Different Analyst	
	DOXH	LVXH	DOXH	LVXH	DOXH	LVXH
1.	100.23	100.68	102.31	100.13	102.51	101.29
2.	101.71	100.48	101.54	101.17	101.35	101.48
3.	100.65	101.36	102.38	100.68	101.48	102.51
4.	102.41	101.51	101.14	100.25	101.64	101.48
5.	101.54	100.18	102.68	101.54	101.27	100.25
<b>Mean</b>	<b>101.30</b>	<b>100.84</b>	<b>102.01</b>	<b>100.75</b>	<b>101.65</b>	<b>101.40</b>
<b>±SD</b>	<b>0.777</b>	<b>0.511</b>	<b>0.575</b>	<b>0.536</b>	<b>0.447</b>	<b>0.718</b>
<b>% RSD</b>	<b>0.767</b>	<b>0.507</b>	<b>0.563</b>	<b>0.532</b>	<b>0.440</b>	<b>0.708</b>

3.6.3. Specificity.

The spectra obtained from the DOXH and LVXH solution were discovered to be identical to those acquired for the standard solution (Fig. 1).

**Table 6.** Specificity analysis at different parameters.

Sr. No.	Parameters	DOXH*	LVXH*
1.	Room Temp.	102.21	101.35
2.	Acid (0.1N HCL)	83.24	74.36
3.	Alkali (0.1N NaOH)	91.51	90.21
4.	Oxide (3% H <sub>2</sub> O <sub>2</sub> )	85.64	87.35
5.	Heat 24hr (60°C)	95.37	92.54

\*mean of three determination

3.6.4. Limit of Detection

The estimation of LOD values of DOXH and LVXH were found to be 1.41 and 0.63 µg/ml, respectively (Table 9).

3.6.5. Limit of Quantitation.

The estimation of LOQ values of DOXH and LVXH were found to be 4.30 and 1.92 µg/ml, respectively (Table 9).

3.6.6. Sensitivity.

Sensitivity analysis shows promising results in terms of method validation. Sandell's sensitivity was discovered to be adequate and acceptable (Table 9).

3.6.7. Accuracy.

Percent recovery for DOXH at recovery level of 80% was found to be 99.7, at recovery level of 100% was found to be 99.66, and at recovery level of 120% was found to be 99.69.

**Table 7.** Recovery analysis of proposed method at three different recovery levels.

Sr. No.	Recovery level	Amount of pure drug added (µg/ml)		Amount of drug estimated (µg/ml)		Percent drug estimated	
		DOXH	LVXH	DOXH	LVXH	DOXH	LVXH
1.	80%	8	8	7.98	7.97	99.75	99.62
		8	8	7.98	7.97	99.75	99.62
		8	8	7.97	7.96	99.62	99.5
		<b>Mean</b>				<b>99.7</b>	<b>99.58</b>
		<b>±SD</b>				<b>0.058</b>	<b>0.058</b>
		<b>% RSD</b>		<b>0.059</b>	<b>0.059</b>		
2.	100%	10	10	9.97	9.96	99.7	99.6
		10	10	9.97	9.96	99.7	99.6
		10	10	9.96	9.98	99.6	99.8
		<b>Mean</b>				<b>99.66</b>	<b>99.66</b>
		<b>±SD</b>				<b>0.047</b>	<b>0.094</b>
		<b>% RSD</b>		<b>0.047</b>	<b>0.094</b>		
3.	120%	12	12	11.96	11.97	99.66	99.75
		12	12	11.96	11.96	99.66	99.66
		12	12	11.97	11.94	99.75	99.5
		<b>Mean</b>				<b>99.69</b>	<b>99.63</b>
		<b>±SD</b>				<b>0.039</b>	<b>0.103</b>
		<b>% RSD</b>		<b>0.039</b>	<b>0.104</b>		

Percent recovery for LVXH at recovery level of 80% was found to be 99.58, at recovery level of 100% was found to be 99.66, and at recovery level of 120% was found to be 99.63 (Table 7). In this method, the amount of the individual drug was estimated by solving the simultaneous equation at 273nm and 287nm using the respective absorptivity value.

**Table 8.** Analysis of laboratory synthetic mixture by the proposed method.

Sr. No.	Amount of drug taken for assay (µg/ml)		Amount of drug estimated (µg/ml)		Percent drug estimated	
	DOXH	LVXH	DOXH	LVXH	DOXH	LVXH
1.	10	10	09.97	09.98	99.7	99.8
2.	10	10	09.98	10.12	99.8	101.2
3.	10	10	10.1	10.11	101	101.1
4.	10	10	10.11	09.97	101.1	99.7
5.	10	10	09.97	10.11	99.7	101.1
Mean					<b>100.26</b>	<b>100.58</b>
±SD					<b>0.646</b>	<b>0.679</b>
% RSD					<b>0.645</b>	<b>0.675</b>

**Table 9.** Optical properties and validation parameters of Doxycycline hyclate and Levofloxacin hemihydrate.

Sr. No.	Parameters	DOXH	LVXH
1.	λ max	273nm	287nm
2.	Beer Lambert's linear Conc. range (µg/ml)	2 - 20	2 - 20
3.	Mean Absorptivity values	588.85	610.97
4.	Sandell's sensitivity	0.294	0.237
5.	Regression equation	y = 0.0511x + 0.054	y = 0.0604x + 0.0053
6.	Correlation coefficient (r <sup>2</sup> )	0.9999	0.9998
7.	Slop (m)	0.0511	0.0604
8.	Intercept (c)	0.054	0.0053
9.	SE of Intercept	0.0069	0.0036
10.	SD of Intercept	0.0219	0.0116
11.	LOD (µg/ml)	1.41	0.63
12.	LOQ (µg/ml)	4.30	1.92
13.	Intraday precision % RSD	0.767	0.507
14.	Interday precision % RSD	0.563	0.532
15.	Precision by different analysisist % RSD	0.440	0.708
16.	Percent Recovery (at 80% level)	99.7	99.58
17.	Percent Recovery (at 100% level)	99.66	99.66
18.	Percent Recovery (at 120% level)	99.69	99.63

Overlain spectra of the DOXH and LVXH depicted an iso-absorptive point observed at 280nm. For method development, two absorbance maxima, i.e., λ<sub>max</sub> 273nm and 287nm, were selected at which DOXH and LVXH show the linear relationship between absorption and concentration with r<sup>2</sup> values of 0.9999 and 0.9998 in a range of 2-20 µg/ml concentration. Correlation coefficients of the regression (r<sup>2</sup>) values are used to validate the linearity of the calibration curves, and a higher value of r<sup>2</sup> indicates the precision and acceptable linearity of the developed method. Lower % RSD values in all precision study parameters were below 1%, which means within a range specified by ICH, which reflects the good, acceptable accuracy, preciseness, and repeatability/reproducibility property of the proposed developed method. The addition of DOXH and LVXH standard solutions for recovery analysis did not change spectra' qualities, which confirms the proposed method's validity. Lower values of LOD & LOQ confirm the acceptable sensitivity of the proposed method. Sandell's sensitivity was discovered to be adequate, and this shows that extremely less measure of the two medications can be

successfully recognized by this technique. Greater recovery values indicate the acceptable repeatability and accuracy of the proposed method.

#### 4. Conclusions

Regression analysis (Linearity and Range) for both the drugs was  $> 0.999$ , indicating the validated method's precision. Repeatability and inter and intra-day precision were studied where %RSD was found to be less than 1 for both drugs. A lower value of LOD and LOQ confirms the sensitivity of the specified method. System suitability, the mean % RSD was found to be less than 1 for both DOXH and LVXH, which was found to be well within the acceptable limit.

All these factors lead to the conclusion that the above-validated method mentioned in this paper for simultaneous estimation of DOXH and LVXH by the spectrophotometric technique can be recommended for regular quality control analysis in the synthetic mixture and their combined pharmaceutical dosage forms, and it was found to be simple, accurate, precise, sensitive, reproducible, economical and rapid. The solvents used for the proposed methods were inexpensive and simple to prepare. This method was adopted to be used in a quality control study for regular drug analysis.

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#### Conflicts of Interest

The authors declare no conflict of interest.

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