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## DEVELOPMENT AND VALIDATION OF UV-SPECTROSCOPIC METHOD FOR THE ESTIMATION OF LYMECYCLINE IN CAPSULE DOSAGE FORM

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### Keywords:

Lymecycline, Antibiotic, Capsule, Beer's law, Linearity

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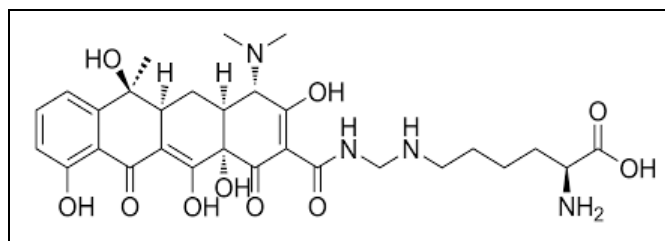
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**ABSTRACT:** Lymecycline is a tetracycline broad-spectrum antibiotic. Present work describes a simple, accurate, precise, economical and reproducible spectrophotometric method in ultraviolet region has been developed and validated for the assay of Lymecycline in bulk and in Pharmaceutical formulations in diluent. Lymecycline is a tetracycline broad-spectrum antibiotic. Lymecycline like other tetracyclines is used to treat a range of infections Lymecycline exhibits absorption maxima at 270 nm in diluent. Beer's law was found to be obeyed in the concentration range of 7.5-22.5 µg/ml. The optimum concentration of the Lymecycline was found to be 15 µg/ml. This concentration of Lymecycline was shown good absorbance values at respective wavelengths was found to be 0.490. Linearity studies were carried out and the range was found to be 7.5-22.5 µg/ml for Lymecycline in diluent. The regression coefficient value of Lymecycline was found to be 0.999 which was not less than 0.995. The method is accurate, precise and economical. In this proposed method, there was no interference from common pharmaceutical excipients. The results of the analysis were validated statistically as per the ICH guidelines. The proposed method was successfully used for the routine analysis of the Lymecycline in bulk and in its capsule dosage form.

**INTRODUCTION:** Lymecycline is a tetracycline broad-spectrum antibiotic. It is approximately 5000 times more soluble than tetracycline base and is unique amongst tetracyclines in that it is absorbed by the "active transport" process across the intestinal wall, making use of the same fast and efficient mechanism by which carbohydrates are absorbed. It inhibits cell growth by inhibiting translation. A literature survey carried out revealed that there is no method reported for estimation of Lymecycline in capsule dosage form by using UV spectroscopy.

Lymecycline is a yellow powder with hygroscopic nature, very soluble in water, practically insoluble in ethanol and methylene chloride. Its melting point is 192.5 °C, Molecular formula C<sub>29</sub>H<sub>38</sub>N<sub>4</sub>O<sub>10</sub>, and having a molecular weight of 602.632 gm/mol, and its chemical names are Limeciclina, Lymecyclinum, N-Lysinomethyl tetracycline, Tetracycline-L-methylene lysine, Tetracycline-L-methylene lysine.



**FIG. 1: STRUCTURE OF LYMECYCLINE**

UV- visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical

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analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. An instrument that measures the ratio, or function of ratio, of the intensity of two beams of light in the UV- visible region is called Ultraviolet-visible spectro-photometers.

This absorption spectroscopy uses electromagnetic radiations between 190 nm to 800 nm and is divided into the ultraviolet (UV, 190-400 nm) and visible (VIS, 400-800 nm) regions.

Since, the absorption of ultraviolet or visible radiation by a molecule leads transition among electronic energy levels of the molecule, it is also often called electronic spectroscopy. The information provided by NMR and IR spectral data leads to valuable structural proposals.

**Method Development:** Method development is the process of proving that an analytical method is acceptable for use to measure the concentration of API in a specific dosage form.

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any pharmacopeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical method may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for the quantitation of the drug in biological fluids may not be available.
- Analytical methods for a drug in combination with other drugs may not be available
- The existing analytical procedure may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures, and these may not be reliable.

Method development should be based on several conditions. It is preferable to have maximum sample information to make development fast and desirable for the intended analytical method, application and physical-chemical properties are most preferable as primary information. Moreover, separation goal needs to define at beginning so; appropriate method can be developed for the purpose. An LC method development is a huge area for even pharmaceuticals with regulatory requirements of international standards. So, before method validation and usage at quality control, many aspects need to focus as per ICH guidelines.

**Method Validation:** Validation of an analytical method is the essential step in the integral process of quality assurance and quality control of chemical measurements in the material systems. According to USFDA, validation is defined as the process a high degree of assurance that a specific process will constantly produce a product meeting its predetermined specifications and quality attribute. The primary objective of validation is to form a basis for written procedures for production and process control which are designed to assure that the drug products have the identity, strength, quality and purity they purport or are represented to process, material, activity or system leads to expected results.

This process consists of the establishment of the performance characteristics and the limitations of the methods. Method validation is required when a new method is being developed. Revision established method. When established methods are used in different laboratories and different analysts.

#### **MATERIALS AND METHODS:**

**Materials:** Lymecycline was obtained as a gift sample from Enaltec Labs Pvt. Ltd. Navi Mumbai Maharashtra, Lymzit 408 mg capsules purchased from Mednear, Diluent as 0.01M HCl, and all other reagents used were of analytical grade.

#### **Methods:**

**UV Spectroscopic Method Development:** UV method was planned to develop for the estimation of Lymecycline. It starts with the choice of the appropriate technique, chromatography, spectroscopy or any other suitable analytical technique.

The following steps were conducted to establish the method conditions for drug substances.

- ✓ The solubility studies.
- ✓  $\lambda_{\max}$  determination.
- ✓ Optimization of concentration of a drug.

**Preparation of Diluent (0.01M HCl):** In 1000 ml volumetric flask containing about 400 ml milli Q water, add 0.9 ml of concentration. HCl swirls to mix to the volume with water

**Preparation of Standard Stock Solution:** Weigh accurately about 100.0 mg of Lymecycline working standard in a 100 ml volumetric flask. Add 50 ml of diluent and mix well, then makeup to the final volume. Further dilution was made by pipetting 0.15 ml of mother liquor into 10 ml volumetric flask and makeup to the volume with solvent. The optimized concentration of the standard was 15  $\mu\text{g/ml}$ . The solution was scanned in UV region in the wavelength range from 200 to 400 nm and  $\lambda_{\max}$  was optimized at 270 nm.

**Preparation of Sample Solution:** Weigh accurately about 100 mg of capsule content into 100 ml volumetric standard flask and add 40 ml of diluent and mix well, then makeup to the final volume. Further dilution was made by pipetting 0.15 ml of mother liquor into 10 ml volumetric flask and makeup to the volume with solvent. The final concentration of the sample was 15  $\mu\text{g/ml}$ . The solution was scanned in the UV region in the wavelength range from 200 to 400 nm, and  $\lambda_{\max}$  was optimized at 270 nm.

**Calculation:** The content of Lymecycline or the percentage purity was calculated using the following formula,

$$\% \text{ Purity} = \frac{\text{Test absorbance} \times \text{Standard dilution} \times \text{Average weight} \times 100}{\text{Standard absorbance} \times \text{Test dilution} \times \text{Labeled claim}}$$

### UV Spectroscopic Method Validation:

#### Precision:

**System Precision:** The system precision was performed by analyzing a standard solution of Lymecycline (15  $\mu\text{g/ml}$ ) at the working concentration level for 6 times.

**Acceptance Criteria:** The percentage relative standard deviation values should be less than 2.0.

**Method Precision:** The method precision was performed by analyzing a sample solution of Lymecycline at a working concentration level for 6 times. Then the precision was confirmed by performing intraday and interday analysis.

**Acceptance Criteria:** The percentage relative standard deviation values should be less than 2.0

**Linearity and Range:** The linearity of an analytical method is its ability (within a given range) to obtain the test results which are directly proportional to the concentration (amount) of analyte in the samples within a given range. A calibration curve was plotted between concentration and absorbance. The linearity was performed in various concentrations ranging from 7.5, 10, 12.5, 15, 17.5, 20, and 22.5  $\mu\text{g/ml}$ . Lymecycline was linear with the concentration range of 7.5-22.5  $\mu\text{g/ml}$  at 270 nm.

**Acceptance Criteria:** The regression coefficient is not less than 0.999.

**LOD and LOQ:** The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average slope and standard deviation of the intercept.

**Accuracy:** The accuracy of an analytical method is the closeness of test results obtained by that method to the true value or an accepted reference value. It is a measure of exactness of an analytical method or the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy is calculated as the percentage recovery by the content of the known added amount of analyte in the sample. The accuracy of an analytical method should be established across the range.

The determination of the content of Lymecycline was performed at three levels by adding the calculated amount of Lymecycline. The sample was prepared in triplicate (9 determinations) *i.e.* 50%, 100%, 150% of the working concentrations for the method.

**Preparation of 50, 100 and 150% Sample Solution:** Weigh accurately about 100 mg of capsule content into 100 ml volumetric standard

flask and add 40 ml of diluent and mix well, then makeup to the final volume. Further dilution was made by taking 1.85, 2.2, and 2.55 ml of mother liquor into three 10 ml volumetric flasks and makeup to the final volume with solvent. The final concentrations of Lymecycline samples were 18.5  $\mu\text{g/ml}$ , 22  $\mu\text{g/ml}$  and 25.5  $\mu\text{g/ml}$  respectively. The solutions were scanned at 270 nm.

**Acceptance Criteria:** 98% to 102% recovery.

**Specificity:** The specificity is the ability to assess the unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. The specificity of the method corresponds to the non-interaction of the placebo with that of the active moiety. The specificity of the method is established by known concentration of Lymecycline is taken in different solvents and estimated as per analytical method.

**Acceptance Criteria:** The percentage relative standard deviation values should be less than 2.0.

**Ruggedness:** The ruggedness of an analytical method is the degree of reproducibility of test results obtained the analysis of the same samples under a variety of conditions such as different laboratories, analysts, instruments, operational conditions. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst. The ruggedness of an analytical method is to be determined by analyzing the standard and sample solution in duplicate using the following

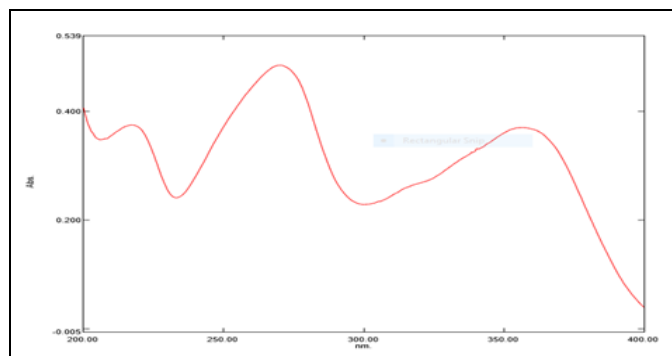


FIG. 2: UV SPECTRUM OF LYMECYCLINE STANDARD

**Standard and Sample UV Spectrum of Lymecycline:** The sample and standard solutions of 15  $\mu\text{g/ml}$  of lymecycline in diluent were

parameters, like different analysts and different days.

**Acceptance Criteria:** The percentage relative standard deviation values should be less than 2.0

**Robustness:** The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of methods was performed at different wavelengths (270  $\pm$  2 nm).

**Acceptance Criteria:** The percentage relative standard deviation values should be less than 2.0

**RESULTS AND DISCUSSION:** Simple, rapid, precise, and accurate UV spectrophotometric method was developed and validated for the estimation of Lymecycline in the capsule dosage form.

**Solubility Profile:** The solubility studies of lymecycline in a number of polar and non-polar solvents were tried to dissolve the drug. From the solubility profile diluent was chosen as common solvent for the estimation Lymecycline. The solubility data were shown in **Table 1**.

TABLE 1: SOLUBILITY PROFILE OF LYMECYCLINE IN DIFFERENT SOLVENTS

S. no.	Solvents	Solubility
1	Distilled water	Very soluble
2	0.01M HCl	Freely soluble
3	Ethanol	Slightly soluble
4	Methylene chloride	Insoluble

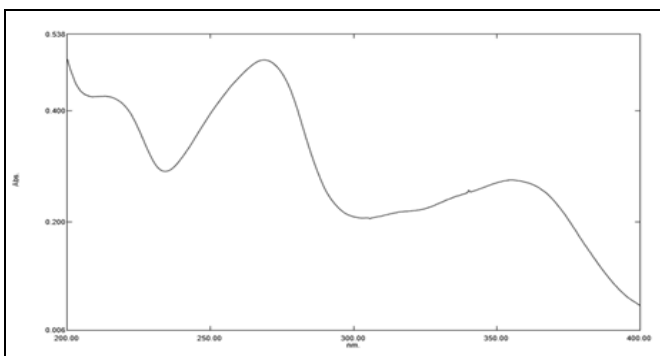


FIG. 3: UV SPECTRUM OF LYMECYCLINE SAMPLE

prepared individually, and the solutions were scanned in UV region in the wavelength range from 200-400 by using diluent as blank and standard and

sample spectrum of lymecycline was shown in **Fig. 2** and **3**. From the spectrum 270 nm was selected for the estimation of Lymecycline without any interference.

**Calibration of Lymecycline:** The optimum concentration of the Lymecycline was found to be 15 µg/ml. This concentration of lymecycline was shown a good absorbance value at 270 nm wavelength was found to be 0.490.

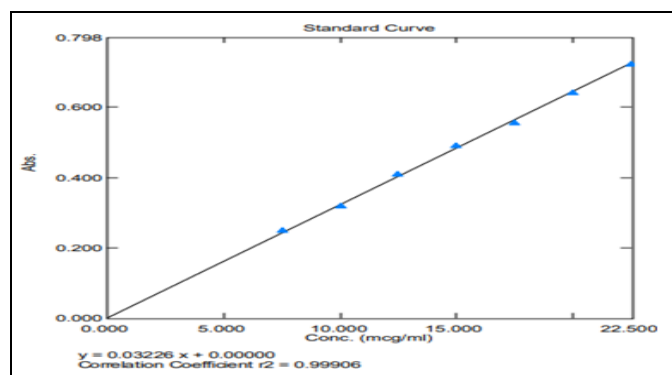
Different aliquots of Lymecycline in diluent were prepared in the concentration. Range of 7.5-25 µg/ml, the absorbance values of solutions was measured at 270 nm and shown in **Table 2**.

**TABLE 2: CALIBRATION DATA FOR LYMECYCLINE**

Concentration	Absorbance
7.5	0.251
10	0.321
12.5	0.410
15	0.490
17.5	0.556
20	0.643
22.5	0.724

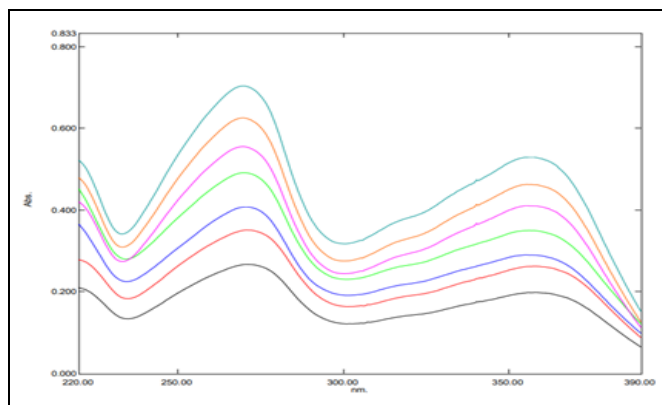
The calibration curve was plotted using concentration against absorbance. The calibration graph at 270 nm for Lymecycline was shown in **Fig. 3**, the preparation of the calibration curve was repeated for 6 times for each solution at their selective wavelengths. The correlation coefficient for the drug solution of different solvents was found to be above 0.9994.

This indicates that all the drug solutions obey beer's law in the selected concentration range. Hence the concentration was found to be linear.



**FIG. 4: CALIBRATION CURVE FOR LYMECYCLINE**

The linearity curves for lymecycline showed the regression coefficient value 0.999 which complies that the limit of not less than 0.993



**FIG. 5: OVERLAY STANDARD SPECTRUM OF LYMECYCLINE FOR LINEARITY STUDY**

**Precision:** The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.46675 in the diluent. The low % RSD values indicate that the method has good precision. The precision was performed by analyzing standard and sample solutions of lymecycline 15 µg/ml at working concentration. Level 6 times. The % RSD values of system precision and method precision were found to be 0.46675 and 0.34475 respectively. The results were shown in **Tables 3** and **4**. The results showed that the precision of the method was confirmed.

**TABLE 3: EVALUATION DATA OF SYSTEM PRECISION STUDY**

S. no.	Standard Absorbance values at 270 nm
1	0.506
2	0.508
3	0.510
4	0.509
5	0.505
6	0.504
Mean	0.507
SD	0.002366
% RSD	0.466751

The percentage relative standard deviation value of standard absorbance for lymecycline in diluent was within the limit, less than 2.0

**TABLE 4: EVALUATION DATA OF METHOD PRECISION STUDY**

S. no.	Sample Absorbance values at 270 nm
1	0.474
2	0.476
3	0.475
4	0.472
5	0.473
6	0.472
Mean	0.473667
SD	0.0016329
% RSD	0.3447554

The percentage relative standard deviation value of sample absorbance for lymecycline in diluent was within the limit, less than 2.0.

Further the precision of the method was confirmed by intra-day and inter-day analysis. The analysis of formulation was carried out for the 3 times in the same day and one time in the 3 consecutive days. The % RSD value of intra-day analysis was found to be 0.1454 (standard) and 0.4133 (sample) for lymecycline 15 µg/ml in diluent. The % RSD value of inter-day analysis was found to be 0.286 (standard) and 1.16 (sample) for lymecycline in diluent. The results were shown in **Table 5** and **6**.

**TABLE 5: INTRADAY PRECISION DATA FOR LYMECYCLINE**

Parameter	Lymecycline absorbance values at 270nm	
	Standard	Sample
Absorbance	0.487	0.482
at $\lambda$ max	0.486	0.484
	0.485	0.487
	Mean	0.486
SD	0.0007	0.0021
%RSD	0.1454	0.4133

The percentage relative standard deviation values of absorbance for lymecycline standard and sample in diluent were the limit, *i.e.*, less than 2.

**TABLE 6: INTERDAY PRECISION DATA FOR LYMECYCLINE**

Parameters	Lymecycline absorbance values at 270 nm			
	Day 1		Day 2	
	Standard Absorbance	Sample Absorbance	Standard Absorbance	Sample Absorbance
Day to day	0.490	0.489	0.495	0.493
	0.489	0.478	0.493	0.492
	0.487	0.478	0.491	0.493
Mean	0.488	0.481	0.493	0.492
SD	0.00141	0.0056	0.00141	0.000707
%RSD	0.286	1.16	0.286	0.1436

The percentage relative standard deviation values of absorbance for lymecycline and sample in diluent were within 2.0.

**Accuracy:** The accuracy of the method was performed by recovery studies. A known quantity of lymecycline raw material solution was added at different levels of 50,100,150%.

The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.71-100.09% w/v for lymecycline in the diluent. The low % RSD value of drug solutions indicates that this method is very accurate. The recovery data was shown in **Table 7**.

**TABLE 7: EVALUATION DATA OF ACCURACY STUDY OF LYMECYCLINE**

% Recovery Level	% Recovery	Mean % Recovery	SD	% RSD
50%	0.327	99.71	0.001	0.30487804
	0.328			
	0.329			
100%	0.509	100.03	0.001	0.1960784
	0.510			
	0.511			
150%	0.751	100.09	0.001	0.1329787
	0.752			
	0.753			

**Acceptance Criteria:** 98% to 102% recovery

**Ruggedness:** The developed data were validated for ruggedness. It refers to the specific of one lab to multiple days which may include analysis, different instruments and different sources of reagents and so on. In the present work, it was confirmed by

analyst. The % RSD value by analyst 1 and analyst 2 was found to be 0.3061 and 0.3196 for lymecycline in diluent at 15 µg/ml.

The low % RSD values indicate that the developed method was more rugged. The results were shown in **Table 8**.

**TABLE 8: RUGGEDNESS DATA FOR ANALYSIS TO ANALYST**

Parameter	Standard			Sample		
	Analyst 1	Analyst 2	Analyst 3	Analyst 1	Analyst 2	Analyst 3
Analyst to	0.488	0.477	0.472	0.488	0.478	0.508
Analyst	0.490	0.479	0.474	0.489	0.479	0.509
	0.491	0.480	0.475	0.490	0.480	0.510
Mean	0.490	0.4786	0.47366	0.489	0.479	0.509
SD	0.001	0.00153	0.001527	0.001	0.001	0.001
%RSD	0.306122	0.319682	0.316656	0.204498	0.208768	0.196463

The percentage relative standard deviation values of absorbance for Lymecycline standard and sample in diluent were within the limit, *i.e.* less than 2.0

**Robustness:** Robustness were performed at a different wavelength by using working standard solutions of lymecycline. The % RSD values for wavelength variation were found to be 0.02016 and 0.04196 at 268, 270 and 272 nm respectively. The low % RSD values indicate that the development method was more rugged. The results were shown in **Table 9**.

**TABLE 9: ROBUSTNESS DATA FOR WAVELENGTH VARIATION**

Wavelength (nm)	Drug	
	Standard	Sample
268	0.495	0.4768
270	0.497	0.4766
272	0.496	0.4764
Mean	0.496	0.4766
SD	0.0001	0.0002
%RSD	0.0201612903	0.0419639110

The percentage relative standard deviation values of absorbance for Lymecycline in standard and sample in diluent at different wavelengths were within the limit, *i.e.*, less than 2.0

**Optical Parameters:** Optical parameters like molar absorptivity, correlation, slope, intercept, LOD, LOQ and standard error were calculated and results were shown in **Table 10**.

**TABLE 10: OPTICAL CHARACTERISTICS OF LYMECYCLINE**

Parameters	Lymecycline in diluent
Beer's Law limit ( $\mu\text{g/ml}$ )	7.5-25
Molar absorptivity ( $\text{LMol}^{-1} \text{cm}^{-1}$ )	0.0338
Correlation coefficient ( $r^2$ )	0.99906
Regression equation ( $y=mx+c$ )	$y=0.03226x+0.0000$
Slope (m)	$R^2=0.99906$
Intercept (c)	0.0000
LOD ( $\mu\text{g/ml}$ )	0.2414
LOQ ( $\mu\text{g/ml}$ )	0.73155
Standard error	0.001668

**CONCLUSION:** Present work describes a simple, accurate, precise, economical and reproducible spectrophotometric method in the ultraviolet region has been developed and validated for the assay of Lymecycline in bulk and Pharmaceutical formulations in diluent. Lymecycline exhibits absorption maxima at 270 nm in diluent. Beer's law was found to be obeyed in the concentration range of 7.5-22.5  $\mu\text{g/ml}$ . The optimum conc. of the Lymecycline was found to be 15  $\mu\text{g/ml}$ . This conc. of Lymecycline was shown good absorbance values at respective wavelengths were found to be 0.490. Linearity studies were carried out, and the range was found to be 7.5-22.5  $\mu\text{g/ml}$  for Lymecycline in diluent. The regression coefficient value of Lymecycline was found to be 0.999 which was not less than 0.995. The method is accurate, precise and economical. In this proposed method, there was no interference from the common Pharmaceutical excipients. The results of the analysis were validated statistically as per the ICH guidelines. The proposed method was successfully used for the routine analysis of the Lymecycline in bulk and in its capsule dosage form.

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**CONFLICTS OF INTEREST:** The authors declare that there is no conflict of interest regarding the publication of this paper.

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