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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF LYMECYCLINE IN BULK AND CAPSULE DOSAGE FORM

Prashant G. Rajput, Sandip D. Firke*, Dipak K. Lokwani and Nikhil K. Randhir

Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India.

***Corresponding Author: Dr. Sandip D. Firke**

Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India.

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ABSTRACT

The Reversed-Phase chromatographic LC method was developed and validated for the estimation of Lymecycline in Bulk and capsule dosage form. The separation was achieved using a Hypersil BDS C18 column (4.6 mm x 150 mm i.d., 5µ). The mobile phase containing Acetonitrile and Water (pH 3 adjusted with orthophosphoric acid) (50: 50 V/V) was pumped at a flow rate of 1 mL/min. The analyte monitored with PDA detector at 359 nm. The method was found to be linear from 10 to 40µg/ml. The retention time for analyte was found at 2.203 min. All the validation parameters were within the acceptance range. The developed method was successfully applied for quantification of the Lymecycline bulk and capsule.

KEYWORDS: The Reversed-Phase chromatographic Lymecycline bulk and capsule.

INTRODUCTION

Lymecycline is a water-soluble combination of Tetracycline and lysine in the presence of formaldehyde.[1][2][3] Yellow colour compound and very hygroscopic powder having pH 8.1 of 1% solution of lymecycline in water.[4] Lymecycline, chemically is (2S)-6-[[[(4S,4aS,5aS,6S,12aR)-4-(dimethylamino) 1,6,10,11, 12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carbonyl]amino] methylamino]-2-aminohexanoic acid (Figure 1). Lymecycline is a tetracycline broad-spectrum antibiotic. Lymecycline is around multiple times more soluble than tetracycline base and it is the special case among amongst the tetracyclines derivatives in that it is absorbed by the "active transport" process across the intestinal wall.[5]

Literature serves revealed that there is no UV spectrophotometric method was available for the estimation of Lymecycline, one HPLC method available for its estimation[6] the current investigate emphasize a simple, sensitive and effective UV-Spectrophotometry method for estimation of Lymecycline in bulk material and capsule. Further, methods were validated as per ICH guidelines.

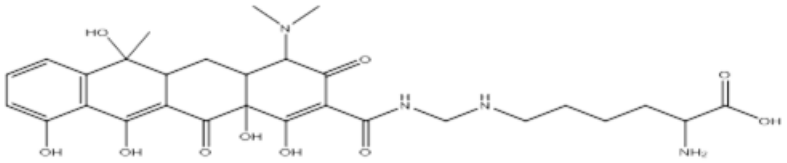

Fig. 1: Chemical structure of Lymecycline.

Experimental

Chemicals and reagents

Lymecycline (91.3% pure) was a gift sample from from Glenmark Pharmaceutical Ltd., Mumbai. HPLC Grade Acetonitrile purchased from Avantor (Rankem), Thane. The double distilled water was used throughout the experiment.

Preparation of solutions

Preparation of Lymecycline Standard Stock solution

Stock solution of Lymecycline was prepared by dissolving accurately weighed 10 mg of the drug in 100 ml of Double distilled water (final concentration, 100 µg/mL). The prepared stock solution was stored at cool temperature and protected from light.

Preparation of Lymecycline sample solution

To estimate the content of Lymecycline in capsules

formulation; Twenty capsules (Involym) were weighed accurately, their content was removed and finely powdered. A quantity equivalent to 10 mg of Lymecycline was weighed and transferred into 100 mL of the volumetric flask containing 50mL of water. The solution was sonicated and filtered through the Whatmann filter paper(no.41) and adjusted volume up to 100 ml to obtain the concentration 100 µL/mL. Aliquot portion 2.5 mL was transferred to 10 mL Volumetric Flask and volume adjusted to mark with the same solvent. The sample solution injected six times into column.

Instrumentation and Chromatographic condition

The HPLC system consisted of Shimadzu (Japan) equipped with a LC-20 AD solvent delivery system (pump), SPD-M20A Photo Diode Array Detector, Data processor LC solution and a Rheodyne injector with 20 µL loop Analysis was carried out at 359 nm using Hypersil BDS C18 (4.6 mm x 150 mm i.d., 5µ) column at 30°C temperature. The mobile Phase consist of Acetonitrile and Water (pH 3 adjusted with ortho phosphoric acid) (50: 50, V/V) that was set at a flow rate of 1.0 ml/min. Prior to injection of drug solution the column was equilibrated for 20 min.

Method Validation

Accuracy/ Recovery studies

To examine the accuracy of the proposed methods and to confirm the interference from excipients used in the formulation, recovery experiments were carried out by adding a known amount of standard drug to marketed capsule formulation at 80, 100 and 120% level. It was then re-analyzed by the proposed methods. The % recovery of noted.

Precision

The precision of the proposed method was determined in terms of intra-day and inter-day precision. Intra-day precision was resolute by examining the 20, 25 and 30 µg/ml of Lymecycline three times in the similar day. Inter-day precision was determined the concentration of 20, 25 and 30 µg/ml of Lymecyline for three days.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were calculated by using the equations designated by International Conference on Harmonization (ICH) guidelines. LOD = 3.3 x σ / S LOQ = 10 x σ /S, Where σ is the standard deviation of intercept, S is the slope.

Linearity

Linearity was performed by using seven concentrations level of analyte ranging between 10-40 µg/ml.

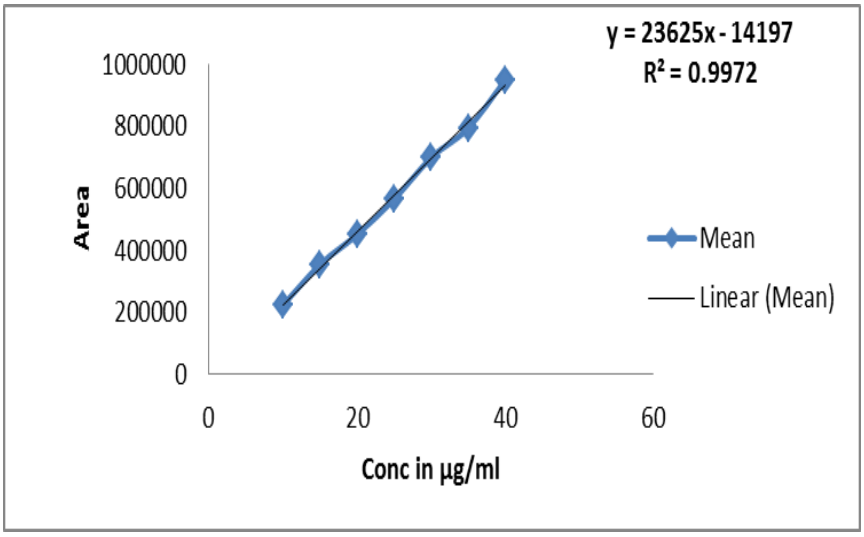

Fig. 2: Linearity curve of Lymecycline.

Ruggedness

The ruggedness of the introduced method was determined for 25 µg/ml concentration of Lymecycline by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions. The results are in the acceptable range that is % RSD values < 2 for all the methods.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The optimization of mobile phase was started with using Methanol and Water in ratio of 50:50% v/v it was observed that the drug was not eluted further the composition of mobile phase was changed but the satisfactory peak not observe then pH of water adjusted 3 with OPA its show the good shape but along with some impurities so mobile phase was revised using ACN : Water pH 3 50:50 v/v with 1 ml/min flow rate it show

the good peak with tailing factor 1.327 and Retention time 2.203 min having capacity factor 1.1.

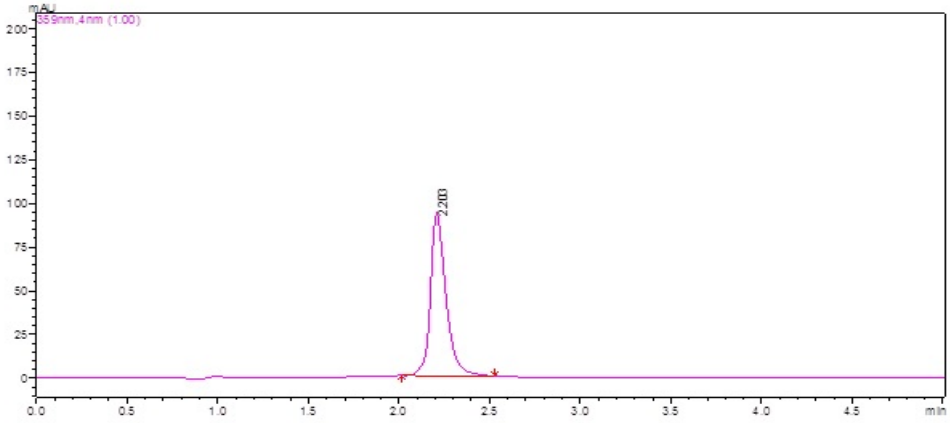

Fig. 3: Optimized Chromatogram of Lymecycline.

Method validation

The developed method was validated in terms of precision, accuracy, limit of quantification (LOQ), Limit of Detection (LOD), specificity, ruggedness and robustness according the international conference on harmonization (ICH) guidelines Q2(B).[7][8]

System suitability

The System Suitability test was performed, the obtained results such as theoretical plate, tailing factor, Retention time Showed in to the Table 1.

Table 1: System Suitability Studies.

Retention time	2.203 min
Capacity factor	1.2
Theoretical plate	3043
Tailing Factor	1.327

Accuracy and Precision

The accuracy of the lymecycline drug was performed by the standard addition method, Where the known amount of standard added in three different levels i.e 80, 100, 120% for marketed capsule formulation of lymecycline and the % recovery and %RSD found within the limit i.e 99.60-100.25%. The results of studies was showed in Table 2.

Table 2: Accuracy.

Drug	Initial Amount [µg/mL]	Excess drug added to the analyte [%]	Total amount found±SD[µg/mL]	Recovery [%] [n=3]	%RSD [n=3]
Lymecycline	15	80	27.03 ± 0.18	100.25	0.66
	15	100	30.02 ± 0.14	100.17	0.48
	15	120	32.92 ± 0.10	99.60	0.30

n- number of determinations

Table 3: Precision Studies [Intra and Inter-day].

Standard Concentration [µg/mL]	Amount found [µg/mL]	% Amount found [µg/mL] [n=3]	% RSD
Intra-day Precision			
20	20.02	99.61	0.10
25	24.72	99.51	0.62
30	29.81	99.22	0.53
Inter-day Precision			
20	19.98	99.66	0.28
25	24.78	99.14	0.23
30	29.92	99.72	0.70

n - number of determination

Table 4: Repeatability.

Standard Concentration [µg/mL]	Area	Amount Found [µg/mL]	% Amount Found
25	567547	24.62	98.50
25	565451	24.54	98.14
25	568172	24.65	98.60
25	566583	24.58	98.33
25	566651	24.59	98.34
25	566457	24.58	98.31
AVERAGE	566810	24.59	98.37
%RSD	0.17	0.16	0.16

Precision of the intended method was carried out in terms of repeatability and intra-day and inter-day variations. The repeatability of method was analyzed by performing six repeat estimations of 25µg/mL; the effects on the results were studied in terms of %RSD and found to be less than 2. Intra-day variation was performed by checking three different analyte concentrations for three times during a same day and Inter-day precision was evaluated by three different analyte concentrations for three back to back different days, intra-day and inter-day variation were measured at three different concentrations 20, 25 and 30 µg/mL. The impact on results of intra-day and inter-day variations were assessed in terms of % RSD; and results were Shown in Table 3.

Limit of Quantification and Limit of Detection

The LOD and LOQ were calculated using equations; LOD= 3.3 × N/B and LOQ = 10 × N/B, where, N is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. The LOD and LOQ for Lymecycline were found to be 0.116 µg and 0.350 µg, respectively. The obtained LOD and LOQ values showed the higher sensitivity to the optimized mobile phase mixture.

Specificity

Specificity study is a practice to measure quantitatively the analyte in existence of constituent that may be likely to be in the sample matrix. The results of specificity study revealed that the there was no other interfering peak around the retention time of drug.

Robustness and Ruggedness

Robustness of the method was established to evaluate the influence of small but purposeful dissimilarity in the chromatographic conditions for the determination of the percentage of Lymecycline. The Robustness of the method was performed at a concentration level of 25 µg/mL. When extremely little changes were made to the technique conditions there were no checked changes in chromatographic and content of the drug. Ruggedness of prposed method was evaluated by using two different analysts under the same experimental and environmental conditions. the results of ruggedness study revealed that value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method

Assay of Marketed Involym Capsule formulation

Assay of *Lymecycline capsule* containing 408 mg of Lymecycline along with common excipients performed at concentration of 25 µg/mL. The amounts of Lymecycline determined were found to be 100.06 An excellent amount of recovery showed that there was no interference from the excipients present in the *INVOLYM* Capsule dosage form. Assay results for a INVOLYM tablet was represented in Table 5.

Table 5: Assay of Marketed Involym Capsule formulation.

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	%Amount found
Lymecycline	25	25.44	101.75
	25	25.11	100.42
	25	24.88	99.54
	25	24.93	99.72
	25	24.73	98.94
	25	25.01	99.97
	Mean ± SD	25.01 ± 0.22	100.06 ± 0.88
	% RSD	0.88	0.88

REFERENCES

1. British Pharmacopoeia, 2002, volume I, Stationery Office, London, 1063-64.
2. Sjolin-Forsberg G, Hermansson J. Comparative bioavailability of tetracycline and lymecycline. British journal of clinical pharmacology, 1984 Oct; 18(4): 529-33.
3. Whitby JL, Black HJ. Comparison of Lymecycline with tetracycline hydrochloride. British medical journal, 1964 Dec 12; 2(5423): 1491.
4. Lightbown JW, Thomas AH, Berryman IL. The second international reference preparation of lymecycline. Bulletin of the World Health Organization, 1973; 48(1): 81.
5. https://pubchem.ncbi.nlm.nih.gov/compound/Lymecycline.
6. Rao TN, Patrudu TB, Rao TS, Parvathamma T. Simple and Sensitive Validated Reverse Phase

HPLC-UV Method for the Determination of Lymecycline in Pharmaceutical Dosage Forms. International Journal of Pharmaceutical Sciences and Research, 2012 Jul 1; 3(7): 2301.

7. Chung Chow Chan, Y.C.Lee, Herman Lam, Xue-Ming Zhang. "Analytical Method Validation And Instrument Performance Verification" John Wiley & Sons, Inc., Hoboken, New Jersey, 27-49.
8. ICH Q2B. Validation of analytical procedure; methodology, federal register, 2005; 1196(60): 27464.