IJPSR (2012), Vol. 3, Issue 07 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 March, 2012; received in revised form 25 June, 2012; accepted 28 June, 2012

SIMPLE AND SENSITIVE VALIDATED REVERSE PHASE HPLC-UV METHOD FOR THE DETERMINATION OF LYMECYCLINE IN PHARMACEUTICAL DOSAGE FORMS

T. Nageswara Rao*[1], T.B. Patrudu [2], T. Srinivasa Rao [3] and T. Parvathamma [1]

Department of Analytical Chemistry, International Institute of Biotechnology and Toxicology (IIBAT) [1], Padappai-601 301, Kanchipuram District, Tamil Nadu, India
Department of Engineering Chemistry GITAM University, Hyderabad Campus [2], Andhra Pradesh, India
Scientist, Johnson & Johnson – Health Care Products & Pharmaceuticals, Mumbai [3], Maharashtra, India

Keywords:
Lymicycline,
RP-HPLC method,
Reverse phase chromatography
Validation

Correspondence to Author:

T. Nageswara Rao

Research Scholar, Department of Analytical Chemistry, International Institute of Biotechnology and Toxicology (IIBAT), Padappai-601 301, Kanchipuram District, Tamil Nadu, India

ABSTRACT

A Simple, Sensitive, Precise, and specific reverse phase high performance liquid chromatographic method has been developed for the determination of lymecycline in pharmaceutical Dosage Forms. Chromatographic separation was achieved on a PLRP-S (250×4.6 mm), 8.0 µm make: Varian column with a 11.5:10:20:1:57.5 mixture of 2-Methyl-2-propanol,3.5% w/v di-potassium hydrogen phosphate, 1.0% w/v Tetra butyl ammonium hydrogen sulphate, 4.0% w/v di -sodium edetate and JT Baker water as mobile phase, detection was at 254 nm. Response was a linear function of concentration in the range 5-0.02 mg/L for lymecycline; correlation coefficient was 0.9998, respectively. LOD and LOQ for lymecycline were found 0.02 mg/L and 0.05 mg/L. Accuracy (recoveries 95-97%) and reproducibility were found to satisfactory.

INTRODUCTION: Lymecycline (**Fig. 1**) (2S)-6-[({[(4S, 4aS, 5aS, 6S, 12As)-4(dimethylamino)-3, 6, 10, 10, 12a-pentahydroxy-6-methyl-1,11-dioxo-1, 4, 4a, 5, 5a6, 11, 12a-octahydrotetracen-2-yl]-formamido}-methyl) amino]-2-aminohexanoic acid is antibiotic work by killing bacteria that to the cause of acane. They also have a direct effect of reducing inflammation. Lymecycline (Tetralysal) [1] is a tetracycline –amino-acid compound obtained by combining tetracycline [2,3,4] base with L-lysine in the presence of formaldehyde. Its formula (de Carneri and Manfredi, 1962) gives a molecular weight for the pure substance of 602.7.

In this paper, we described a simple, sensitive, and validated RP-HPLC method for determination of lymecycline in pharmaceutical Dosage Forms. The method has been successfully used for quality control analysis of the drugs and other analytical purposes.

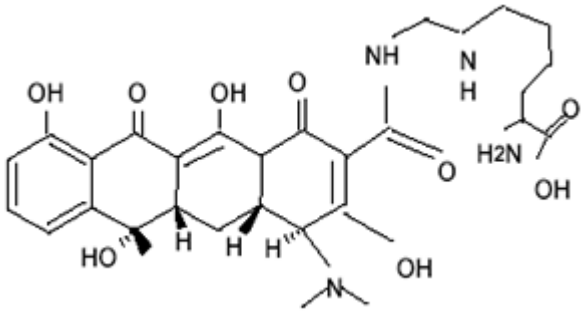

FIG. 1: STRUCTURE OF LYMECYCLINE

Apparatus and Chromatographic Conditions: Chromatographic separation was performed on a Shimadzu chromatographic system equipped with a LC-20AT pump and SPD-20A UV-VIS detector with 20µL fixed loop and analyzed by using LC-Solution software. PLRP-S (250×4.6 mm), 8.0 µm make: Varian column used for separation. Mobile phase consisting of a mixture of 11.5: 10: 20: 1: 57.5 mixture of 2-Methyl-2-propanol, 3.5%w/v di-potassium hydrogen phosphate,

1.0%w/v Tetra butyl ammonium hydrogen sulphate, 4.0%w/v di-sodium edetate and JT Baker water was delivered at flow rate 1ml/min. The mobile phase was filtered through 0.45µ membrane filter and sonicated for 10min. An external standard method was used. UV detection was performed at 254nm and column oven temperature is 50°C. Peak was confirmed by comparison of spectra and retention time with standard.

Reagents and solutions:

Preparation of Diluent (0.01 M Hydrochloric acid): In 1000 ml volumetric flask containing about 400ml milliQwater, added 0.9ml of concentrated Hydrochloric acid swirls to mix to the volume with water.

Preparation of Standard Solution: Accurately weighed 1.02mg of reference standard of Lymecycline in 100ml volumetric flask and the volume was brought upto the mark using diluent.

Preparation of Sample Solution One tablet, containing 408mg of lymecycline was weighed accurately and transferred to a 100 ml volumetric flask with 30ml diluent, shaken for 5min, and then diluted to volume with diluent to furnish a solution containing 408 mg/L lymecycline. After filtration the solution the solution was diluted with diluent to give a final concentration of 10 mg/L lymecycline.

Method Validation: Once the HPLC method development was over, the method was validated in terms of parameters like specificity, press ion, accuracy, linearity and range, LOD, LOQ, raggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values ware calculated. The proposed HPLC method was validated as per ICH guidelines [5, 6, 7].

Linearity and Range: Different known concentrations of lymecycline (5.0 mg/L – 0.02 mg/L) were prepared in diluent by diluting the stock solution. Injected the standard solutions and measured the peak area. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of the method was determined from the correlation coefficient. The results were shown in (**Table 2)**.

The slope, intercept and correlation coefficient values were found to be 28091, 23.54 and 0.9998.

Precision: Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise. Results were shown in (**Table 3-4)**.

Accuracy: To study the reliability, suitability and accuracy of the method recovery experiments were carried out. A known quantity of the pure drug was added to the preanalysed sample formulation at the level of 50% and 100%, dissolved in diluents and made up to 100ml with same solvent. Further dilutions were made so that the each aliquot contained 0.05mg/L of lymecycline. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The recovery procedure was repeated 10 times and % RSD was calculated by using the following formula. The contents of lymecycline per tablet found by proposed method are shown in **(**Table 3); the lower values of RSD of assay indicate the method is accurate. The mean recoveries were in range of 95-97 % which shows that there is no interference from excipients **(Table 5)**.

$$\% \text{ recovery} = \frac{b - a}{c}$$

Where, a-The amount of drug found before the addition of standard drug, b-The amount of drug found after the addition of standard drug, c- The amount of standard drug added

Repeatability of Solution: A standard solution of drug substance was injected ten times and corresponding peak areas were recorded. The % RSD was found to be less than 1 **% (Table 6)**.

Specificity: Condition of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc, was changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peaks shapes.

Assay: 20µl of standard and sample solutions were injected into an injector of RP-HPLC, from the peak area of standard amount of drug in sample were computed. The values are given in **Table 7.**

Limit of Detection and Limit of Quantification: The limit of Detection (LOD) and limit of Quantification (LOQ) of the development method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for lymecycline found to be 0.02mg/L The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10) The LOQ was 0.05mg/L for lymecycline. It was concluded that the developed method is sensitive.

Ruggedness and Robustness: The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC and Agilent HPLC by different operators using different columns of similar types. The %RSD values with two different instruments Shimadzu HPLC and Agilent HPLC, analyst and columns were 0.5-0.5, 0.6-0.5 and 0.4-0.3% respectively. Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in mobile phase, flow rate and column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method is rugged and robust. The robustness limit for mobile phase variation, flow rate variation, and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%.

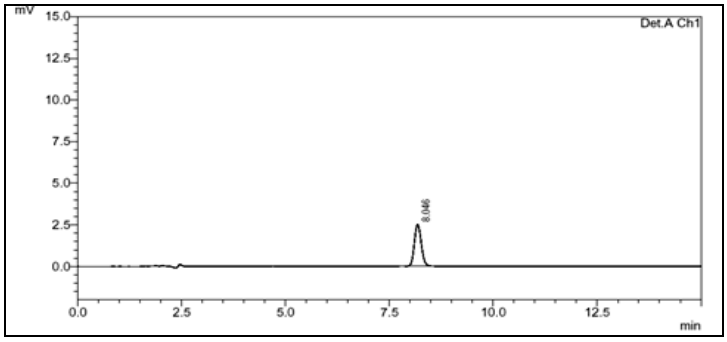

FIG 2: CHROMATOGRAM OF STANDARD (1.0 mg/L)

RESULTS AND DISCUSSIONS: UV spectrum of lymecycline was recorded from which 254nm was selected as wavelength. Flow rate of 1ml/min was selected 11.5: 10: 20: 1: 57.5 mixture of 2-Methyl-2-propanol, 3.5%w/v di-potassium hydrogen phosphate, 1.0%w/v Tetra butyl ammonium hydrogen sulphate, 4.0%w/v di-sodium edetate and JT Baker water was selected as mobile phase. The retention time was found to be 8.0min.Lymecycline shown linearity in the range of 0.02-5mg/L and the co-efficient was found to be 0.9998.

Recovery studies were performed at 50 and 100% levels. The sensitivity of method LOD and LOQ is shown in **(Table 2).** The stability at room temperature and refrigeration was found to be 3 and 8.5 hrs respectively. Hence the proposed method is simple, accurate, and rapid and can be employed for routine analysis. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

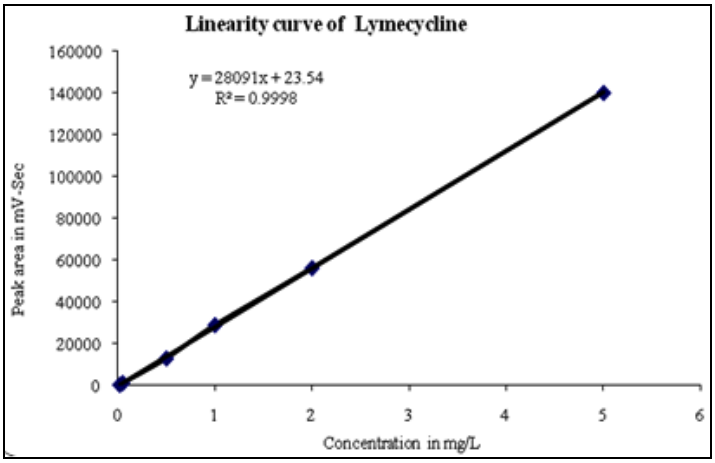

FIG 3: LINEARITY CURVE OF LYMECYCLINE

Regression analysis of the calibration curve for lymicycline showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.9998 in all curves assayed.

The intraday precision was found to be within 1% RSD for conc.0.05, 0.5, 1.0mg/L. Intraday precision was performed for conc. of 0.05, 0.5 and 1.0 mg/L. For about three days and their peak, areas are shown in the **(Table 4)**. The %RSD for conc. 0.05, 0.5, and 1.0 mg/L was found to be within 2%. Recovery studies were performed at 0.05mg/L and 0.5 mg/L levels and the results were found to be within the limits mentioned as per ICH guidelines.

Repeatability of injection was performed using 0.5mg/L sample for 10 times and corresponding peak areas were recorded. The % RSD peak was reported.

Analysis of formulation was performed using Lymecycline 408 mg tablets and the claim was found to be 98.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Parameter	Optimized condition
Chromatograph	HPLC (Shimadzu system equipped with LC-20 AT pump and SPD-20A interfaced with LC Solution software
Column	PLRP-S (250×4.6) mm, 8µ, make: Varian
Mobile Phase	11.5:10:20:1:57.5 mixture of 2-Methyl-2-propanol,3.5% w/v di-potassium hydrogen phosphate,1.0% w/v Tetra butyl ammonium hydrogen sulphate,4.0% w/v di –sodium edetate and JT Baker water
Flow Rate	1.0mL/min
Detection	UV at 254nm
Injection Volume	20µL
Column Oven Temperature	50° C

TABLE 2: VALIDATION PARAMETERS

Parameters	Lymecycline
Linearity range	0.02-5 mg/L
Correlation coefficient	0.9998
Slope	28091
Y Intercept	23.54

TABLE 3: INTRADAY PRECESSION

Concentration (mg/L)	Area	%RSD
0.05	1325	0.87
	1342	
	1349	
0.5	13845	0.36
	13798	
	13897	
1	28845	0.31
	28952	
	29021	

TABLE 4: INTERDAY PRECISION

Concentration (mg/L)	Day	Area	% RSD
0.05	1	1268	1.33
	2	1302	
	3	1289	
0.5	1	12745	1.12
	2	12985	
	3	13004	
1	1	27945	1.21
	2	28612	
	3	28414	

TABLE 5: RECOVERY STUDIES

Level (mg/L)	% Recovery	% RSD
0.05	95	0.1
0.5	97	0.12

TABLE 6: REPEATABILITY OF INJECTION

Conc. (mg/L)	Peak area	%RSD
0.5	13211	0.9049
	13054	
	12998	
	13114	
	13441	
	12921	
	13321	
	13009	
	12899	
	13221	

TABLE 7: ANALYSIS OF FORMULATION

Amount of drug (mg/tab)		% Label claim	%RSD
Labeled	Estimated		
408 mg	0.400	98	0.07

CONCLUSION: A convenient and rapid RP-HPLC method has been developed for estimation of Lymecycline in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of Lymecycline in dosage form, bulk drugs as well as for routine analysis in quality control.

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How to cite this article:
Rao N T., Patrudu T.B., Rao ST and Parvathamma T.: Simple and sensitive Validated Reverse Phase HPLC-UV method for the determination of lymecycline in Pharmaceutical Dosage Forms.. *Int J Pharm Sci Res*. 2012; Vol. 3(7): 2301-2305.

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