International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.7, pp 399-406, 2016

Analytical Quality by Design Approach for Development of UV-Spectrophotometric Method in the Estimation of Lamivudine from Tablet Dosage Form

Sk. Mastanamma*, P. Saidulu, L. Prathibha

Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjunanagar, Guntur–522510, Andhra Pradesh (India)

Abstract : Objective: The aim of present work is to develop and validate spectrophotometric method for lamivudine estimation in tablet dosage form using Analytical Quality by Design (QbD) approach as per ICH Q8 (R2) guidelines.
Methods: Variable parameters like type of sample preparation, solvent, wavelength, instrumental parameters such as slit width, scan speed and sampling interval etc. were designed into Ishikawa diagram and critical parameters were determined by observation as well as by using principal component analysis.
Results: In simple spectrophotometric method lamivudine was estimated at 270 nm using 0.01N NaOH and distilled water at 280nm using 0.01N HCl. Beer's law was obeyed in the concentration range 2-10 µg/ml (r^2=0.998) using 0.01N NaoH 2.5-17.5µg/ml (r^2=0.996) Using distilled water and 2-12 µg/ml (r^2=0.998) using 0.01N HCl.
Conclusion: The proposed method was found to be accurate, precise and economical and can be applicable for routine quality control analysis lamivudine on pharmaceutical dosage form. Implementation of QbD approach resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process and also save time and money.
Keywords: Quality by Design (QbD), lamivudine, ICH Q8 (R2), Principal component analysis.

Introduction

Analytical quality by design (A QbD). As per ICH, QbD is defined as "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management[1-2].

Pharmaceutical industry has focused on product Quality, Safety, and Efficacy. Product quality has been increasing by implementing scientific approaches Will provide the clear and sufficient Knowledge from product development to manufacturing. These QbD tools will minimize the risk by increasing the productivity and quality. Now a days QbD approach has been successfully implemented in generic formulation development. USFDA has released specific QbD guidance for immediate and extended release drug products. Regulatory authorities are always recommending the implementation of ICH quality guidelines Q8 to Q11[3-7].

Equivalent to process QbD, the outcome of a Q b D is well understood and fit for intended purpose with robustness throughout the life cycle has different tools such as ATP (analytical Target profile), CQA, Risk Assessment, Method optimization and development with DoE, MODR (method operable design region),

control strategy and risk Assessment, A Q b D method validation, and continuous method monitoring represents the A QbD life cycle with each tool.

Scientific QbD Approach for Synthesis and analysis ICH Q11 has explained the QbD approach for API synthetic process development but there is no specific discussion on A QbD. However, it is recommended to implement Q b D approach in analytical method development termed as A Q b D. these two specific approaches (QbD and A QbD) can be progressed in equal time represents the necessary steps in API synthesis and analytical development with QbD implementation. This simultaneous implementation produces high quality product.it may give better input for initiation of process analytical technology (PAT).

The expression of tools in QbD is different for synthetic development and analytical development. Both QbD and A QbD tools are presented.

The present work deals with estimation of Lamivudine in tablets using analytical QbD approach in bulk and its tablets dosage forms. Chemical structure of lamivudine shown in fig.1.

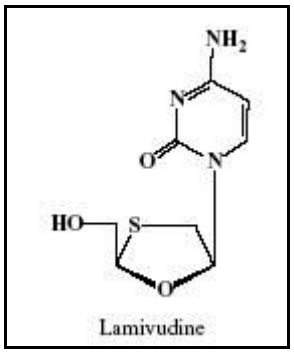


Fig: 1 chemical structure of lamivudine

QbD Approach-

ICH Q8 (R2) guideline introduces a concept of Quality by Design which is defined as - "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management"

Materials and Methods

Instrumentation:

Shimadzu UV -1800 double beam spectrophotometer with 1cm path length supported by shimadzu UV-probe software, version 2.21 was used for spectral measurements with 10mm matched quartz cells. Shimadzu balance (BL-220H) was used for weighing.

Chemicals:

Lamivudine, distilled water, 0.01Nsodium Hydroxide, 0.01Nhydrochloric acid.

Solvents

The ideal property of a solvent is that the drug should be completely soluble in the solvent used and should give constant results. To serve this purpose, three solvents were tried that are 0.01 NHCL, 0.01 N NaOH and distilled water the spectrum was sharp on 0.01N NaOH.

Preparation of stock standard and working solution

Distilled water:

A stock solution was prepared by weighing 10 mg of lamivudine in 10 ml volumetric flask and dissolved in Distilled water to obtain a concentration 1000 µg/ml. Working solution (100 µg/ml) was prepared by diluting 2.5 ml of stock solution to 25 ml and it was used for initial spectral scan in spectrophotometric method and further dilutions for linearity were prepared from the working standard solution by alligation method.

0.01N NaoH:

A stock solution was prepared by weighing 10 mg of lamivudine in 10 ml volumetric flask and dissolved in 0.01 N sodium hydroxide to obtain a concentration 1000 µg/ml. Working solution (100 µg/ml) was prepared by diluting 1 ml of stock solution to 10 ml and it was used for initial spectral scan in spectrophotometric method and further dilutions for linearity were prepared from the stock solution by alligation method.

0.01N HCl:

A stock solution was prepared by weighing 10 mg of lamivudine in 10 ml volumetric flask and dissolved in 0.01M to obtain a concentration 1000 µg/ml. Working solution (100 µg/ml) was prepared by diluting 1 ml of stock solution to 10 ml and it was used for initial spectral scan in spectrophotometric method and further dilutions for linearity were prepared from the stock solution by alligation method.

Determination of detection wavelength for Lamivudine API

Appropriate dilutions of the standard drug solutions were prepared for 10 ppm of lamivudine API. 10 ppm solution of lamivudine was prepared in Distilled water,0.01N NaOH, and 0.01NHCL as a diluent. Solution was scanned using double beam UV VIS spectrophotometer between the range of 200 to 400 nm. λmax of 270nm for distilled water, 0.01Nsodium Hydroxide, 280nm for 0.01 NHCL were considered for experimental work. The UV spectrums are shown in fig.2.

Spectrophotometric conditions

Define method intent:

The goals of HPLC method development have to be clearly defined, as pharmaceutical QbD is a systematic, scientific, risk based, holistic and proactive approach that begins with predefined objectives and emphasizes product and process understanding and control. The ultimate goal of the analytical method is to identify and quantify the main compound.

Step 2: Perform experimental design (1724)

A systematic experimental design is needed to assist with obtaining in depth method understanding and performing optimization. Here an efficient and comprehensive experimental design based on systematic scouting of all three key components of the UV spectrophotometric method (slit width, scan speed and sampling interval) is presented. It forms a spectral analysis that will assist with method understanding, optimization, and selection. In addition, it can be used to evaluate and implement change of the method, should it be needed in the future, for example should the solvent and instrument model used no longer be commercially available. The scoutings of three parameters are shown in table 2. An experimental design comprised of a standard set of 3 solvents, 6 wave length and 3 scan speed was developed. This led to a total of 40 (3 solvents, 6 wave length and 3 scan speed) spectrophotometric conditions.

Step 3: Evaluate experimental results and select final method conditions (2529)

The 40 method conditions were evaluated using the three tiered approach. At the first level, the conditions were evaluated for. % assay . This resulted in 20 spectral conditions for API. At the second level,

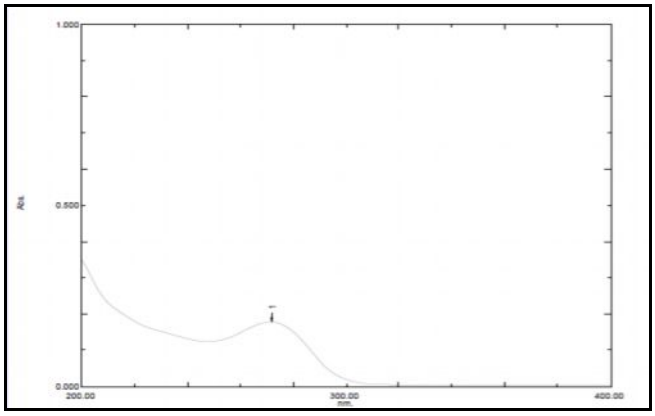
these 20 conditions were further evaluated by using more stringent criteria, such as band pass effect should be less then +nm.

Step 4: Perform risk assessment with robustness and ruggedness evaluation (2933)

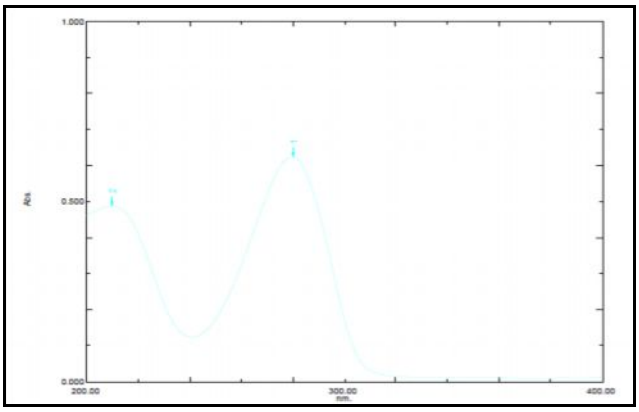
As the final method is selected against method attributes, it is highly likely that the selected method is reliable and will remain operational over the lifetime of product. Therefore, the evaluation of method robustness and ruggedness to be carried out as the fourth step of method development is mainly for the method verification and finalization. A risk-based approach based on the QbD principles set out in ICH Q8 and Q9 was applied to the evaluation of method robustness and ruggedness. Structured methodologies for risk assessment, such as Fishbone diagram can be implemented to identify the potential risk of the method due to a small change of method parameters or under a variety of conditions such as different laboratories, analysts, instruments, reagents, days, etc.

Linearity Studies

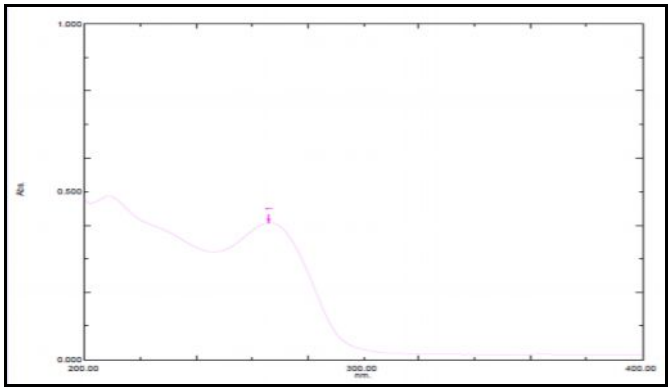
Lamivudine obeyed beer's law in the concentration range of 2–10 µg/ml at 270 nm using 0.01N NaOH. The absorbance was linear in the concentration range of 2–12 µg/ml at 280 nm in 0.01 M HCL The absorbance was plotted against the corresponding concentrations to obtain the calibration graphs (fig.3)**.**



Spectrum of lamivudine in distilled water

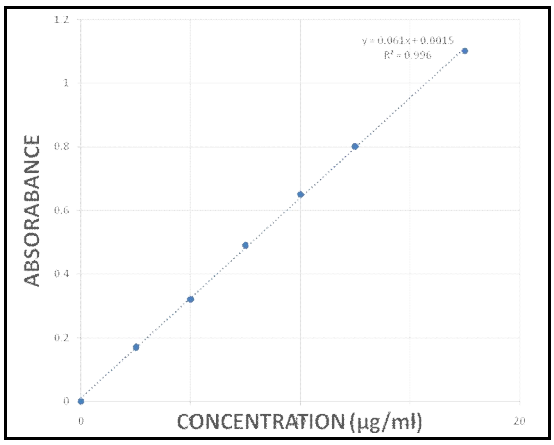


Spectrum of lamivudine in HCL

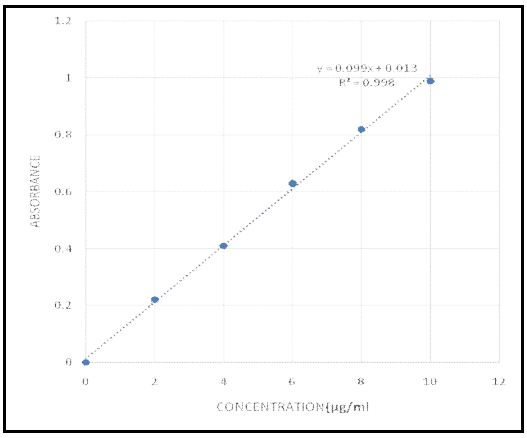


Spectrum of lamivudine in NaOH

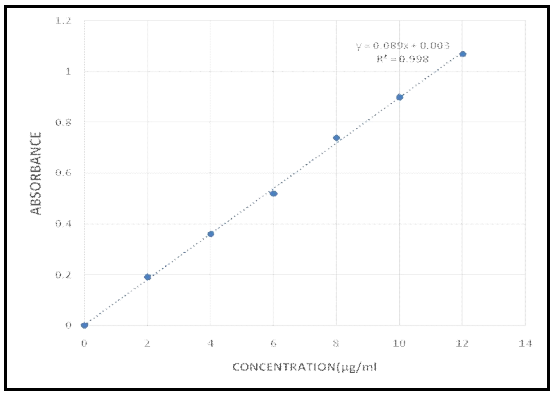
Fig.2. the UV spectrums of lamivudine



Distilled water



0.01N Sodium hydroxide



0.01N Hydrochloric acid

Fig.3 Calibration curves of lamivudine in Different solvents

Estimation of lamivudine in synthetic mixture and tablet dosage form

Lamivudine was estimated by preparing synthetic mixture of label claim 100 mg and it was diluted to 1000 µg/ml. For the estimation in formulation, twenty tablets were weighed and average weight was noted. Then the tablets were triturated and tablet powder equivalent to average weight was weighed and transferred into three 10 ml volumetric flask and diluted with 0.01 N sodium hydroxide, HCl and distilled water. It was shaken for 5 minutes followed by immediate filtration and volume was adjusted up to 10 ml. From this solution, 2.5 ml was withdrawn and further diluted up to 25 ml using 0.01 N sodium Hydroxide, HCl and distilled water. This concentration 100 µg/ml was used for the estimation of further dilution. Finally, percentage amount was calculated.

Table No .1: Result of Marketed Formulation Analysis

Method	**Label claim (mg/tablet)**	**Test concentration (µg/ml)**	**Concentration found (µg/ml) n=6**	**% of assay**
Distilled water	100	8	7.82	97.75
0.01NHCl	100	10	9.92	99.20
0.01 N sodium hydroxide	100	8	7.89	98.6

Validation of proposal methods

Accuracy

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed method, recovery studies were carried out in different recovery levels (50%, 100% and 150%) by adding placebo to the pre-analyzed formulation. The solutions were suitably diluted in the range and then each of the dilution was observed 6 times.

Table 2: results of accuracy studies

S. No	Name of the Drug	Solvent	Pre analysed Concentration (µg/ml)	Recovery Level (%)	Amount added	Amount found	% Recovery
1	Lamivudine	Distilled water	5 5 5	50 100 150	2.5 5 7.5	7.13 10.08 11.8	95% 100.8% 94%
2	Lamivudine	0.01N NaOH	4 4 4	50 100 150	2 4 6	5.28 7.18 9.19	88% 89% 91%
3	Lamivudine	0.01N HCL	4 4 4	50 100 150	2 4 6	5.93 7.61 9.64	98% 95% 96.4%

Precision

Precision studies were performed by using standard solutions containing three concentrations that are 2, 4 and 6 µg/ml for 0.01NNAOH and 2, 4 and 6 µg/ml for0.01N HCL 2.5, 5, 7.5 µg/ml for distilled water.

Repeatability

The precision of the methods in terms of repeatability was determined by analysing three concentrations per three replicates of lamivudine standard solutions. Depending on absorbances obtained for each concentration, standard deviation and percentage relative standard deviation was calculated.

Intermediate precision

Intermediate precision was assessed by analysing lamivudine standard solutions on three consecutive days over a period of one week. Same parameters were calculated for intermediate precision. The results of the repeatability and intermediate precision are as shown in Table 3.

Table 3: Precision data Method Concentration Repeatability

S.No	Solvent	Concentration (µg/ml)	Mean (x)	SD	RSD	LOD & LOQ
1	Distilled water	2.5 5 7.5	0.149 0.354 0.491	0.128 0.016 0.113	0.85 0.045 0.23	0.12 & 0.39
2	0.01NNaOH	2 4 6	0.244 0.472 0.680	0.154 0.0049 0.151	0.63 0.010 0.222	0.23 & 0.61
3	0.01NHCl	2 4 6	0.213 0.378 0.568	0.122 0.0056 0.128	0.572 0.014 0.225	0.18 & 0.56

The limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations 1 and 2.

$LOD = 3.3 \times \sigma / S$…….. (1)

$LOQ = 10 \times \sigma / S$ …….. (2)

Where σ is the standard deviation of intercept, S is the slope of the calibration curve**.**

The results were shown in table 4.

Robustness

Robustness was carried out by changing the concentration of the solvent, the absorbance was taken by using Distilled water, 0.01 N NaoH,0.01 N Hcl as a solvent.

Results and Discussion

The spectrophotometric methods were proposed according to QbD approach. The statistical tool known as principal component analysis was utilized for extraction of critical parameter

Table 4: summary of lamivudine results

S.no	Parameter	Name of the solvent		
		Distilled water	**0.01N NaOH**	**0.01N HCL**
1.	λ max (nm)	270nm	270nm	280nm
2.	Linearity range (µg/ml)	2.5-17.5	2-10	2-12
3.	Regression equation	Y=0.061x+0.015	Y=0.099x+0.0138	y= 0.0895x + 0.0032
4.	Correlation coefficient	0.996	0.998	0.998
5.	Percentage recovery	96..33%	90%	96%
6.	LOD	0.12	0.23	0.18
7	LOQ	0.39	0.61	0.56
8.	Precision (% RSD)	0.37	0.28	0.26

The proposed method was applied to tablet dosage form and amount of drug estimated was 102 % showing good agreement with the label claim. Precision data showed % RSD value less than 2 for repeatability as well as intermediate precision. Accuracy studies showed mean percentage recovery 98.8%. Specificity studies resulted in null interference of excipients with the drug. Limit of detection and limit of quantitation was found to be within limit. Changing in concentration of solvent does not affected the absorbances. The statistical data of validation is summarized in Table 4.

Conclusion:

The proposed spectrophotometric methods can be concluded as accurate, precise, robust, specific and economic. In comparison, the developed spectrophotometric method was found to be more sensitive than simple spectrophotometric method. Implementation of QbD approach resulted in more robust methods with less time consumption, consistency, reliability and quality data.

Acknowledgment

Authors are very thankful to principal, A. Prameela Rani University College of pharmaceutical sciences, Acharya Nagarjuna University, Guntur, for providing the library facilities for literature survey to carryout entire study. Authors also thankful to Hetero laboratories, Hyderabad, India for providing Lamivudine working standard as gift sample.

References

1. "ICH Q8 Quality guidance: Pharmaceutical Development".
2. "ICH Q9 Quality guidance: Quality Risk Management".
3. "ICH Q10 Quality guidance: Pharmaceutical Quality System".
4. "ICH Q11 Quality guidance: Development and Manufacture of Drug Substance".
5. P Borman, et al. The application of quality by design to analytical methods. Pharmtech 2007; 1(2):177–83.
6. M Pohl, M Schweitzer, G Hansen. "Implications and opportunities of applying the principles of QbD to analytical measurements." Pharm Techno Eur 2010; 22(2):29–36.
7. BritishPharmacopoeia2012, British pharmacopeia commission, The department of health, social services and public safety: published by The Stationary Office, London; 2012; 2:2237.

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