# "QbD APPROACH TO ANALYTICAL RP-HPLC METHOD DEVELOPMENT AND ITS VALIDATION

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## ABSTRACT

Quality by design (QbD) refers to the achievement of certain predictable quality with desired and predetermined specifications. A very useful component of the QbD is the understanding of factors and their interaction effects by a desired set of experiments. The present study describes the development of a comprehensive science and risk based HPLC method and subsequent validation for the analysis of zidovudine active pharmaceutical ingredient (API) using a quality by design approach. An efficient experimental design based on systematic scouting of all three key components of the RP-HPLC method (column, pH and mobile phase) is presented. The described method was linear. ($r^{(2)}$=0.9998). The precision, ruggedness and robustness values were also within the prescribed limits (<1% for system precision and <2% for other parameters). Chromatographic peak purity results indicated the absence of co-eluting peaks with the main peak of zidovudine.The proposed method can be used for routine analysis of zidovudine in quality control laboratories.

**Keywords:** Quality by design, HPLC, Zidovudine, Design approach, Quality control labotories.

## INTRODUCTION

Quality by Design (QbD)[1-3] is a concept first outlined by well-known quality expert Joseph M. Juran in various publications, most notably Juran on Quality by Design.While Quality by Design principles have been used to advance product and process quality in every industry, and particularly the automotive industry, they have most recently been adopted by the U.S. Food and Drug Administration[4-7] (FDA) as a vehicle for the transformation of how drugs are discovered, developed, and commercially manufactured. Since first initiated by the U.S. Food and Drug Administration (FDA) in its "Pharmaceutical cGMPs for the twenty-first century" , Quality by Design (QbD) has become an important concept for the pharmaceutical industry that is further defined in the International Conference on Harmonisation (ICH) guidance on pharmaceutical development 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". The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified. The difference between Current approach and Qbd approach is shown in Table 1.

**Table 1: Difference between current approach and Qbd approach**

| Current approach | QbD Approach |
|---|---|
| 1) Quality is assured by testing and inspection. | 1) Quality is built into product & process by design and based on scientific understanding. |
| 2) It includes only data intensive submission which includes disjointed information without "big picture". | 2) It includes Knowledge rich submission which shows product knowledge & process understanding. |
| 3) Here, any specifications are based on batch history. | 3) Here, any specifications based on product performance requirements. |
| 4) Here there is "Frozen process," which always discourages changes. | 4) Here there is Flexible process within design space which allows continuous improvement. |
| 5) It focuses on reproducibility which often avoids or ignores variation. | 5) It focuses on robustness which understands and control variation. |

High-performance liquid chromatography (HPLC)[8-12], particularly Reversed Phase HPLC (RP-HPLC), is the most popular analytical technique in the pharmaceutical industry. The quality of HPLC methods has become increasingly important in a QbD environment. For the purpose of QbD for HPLC methods, robustness and ruggedness should be verified early in the method development stage to ensure method performance over the lifetime of the product. Otherwise, if a non-robust or non-rugged method is adapted, significant time and resource may be required to redevelop, revalidate and retransfer analytical methods.

According to literature survey, there are quite a few publications on HPLC method development strategy but the method development approaches for RP-HPLC specifically focused on pharmaceutical development in a QbD environment have not been widely discussed. Therefore, there is an unmet need to develop a systematic HPLC method development approach for pharmaceutical development using QbD principles to ensure the quality of the method throughout the product lifecycle.

The **aim** of the analytical method is to separate and quantify the main compound while meeting the method performance criteria based on regulatory requirements, such as specificity, linearity, accuracy, precision, sensitivity, robustness, and ruggedness.

The primary objective of this study was to implement Qbd approach to develop and validate an RP-HPLC method that could separate drug from its potential related substances and to establish an in-depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product.

The objectives of this work are as follows:

1. To develop simple, rapid and sensitive method for identification of critical attributes by QbD approach of this antiretroviral drug by RP-HPLC.
2. To establish a validated test method as per ICH guidelines for the determination of assay of this antiretroviral drug by RP-HPLC.

## EXPERIMENTAL

### Materials and reagents

Zidovudine Active Pharmaceutical Ingredient (API) was obtained from Zydus Research Centre. HPLC grade acetonitrile and methanol were purchased from Merck. Potassium Dihydrogen Ortho Phosphate, AR grade Ortho-Phosphoric Acid were purchased from Merck. HPLC grade water was obtained from Milli-Q water purification system (Millipore, Milford, USA).

**Instrumentation and software**

A HPLC (PerkinElmer, Model: Lambda 25) consisting of P.E. Binary LC Pump 200B/250 (PerkinElmer, Model: series 200), vacuum degasser, UV-VIS detector (PerkinElmer, Model: series 200), C8 and C18 reverse phase column (Phenomenex, size: 250×4.60 mm, particle size 5μm) and a sample injector system (Rheodyne) with a 20μl sample loop and Total Chrome Navigator software (version V 6.3.1) on computer (operated with Windows 2003 professional); Vacuum filtration assembly (Orchid Scientifics, Model: JVP01); Ultrasonicator (Oscar, Model: Microclean 103)

**Chromatographic system**

The chromatographic column used was C-18 250mm×4.6mmcolumn with 3 μm particles of Thermo scientific make. The mobile phase consists of methanol (solvent A), and buffer pH 3 (solvent B). The flow rate of the mobile phase was kept at 1.0 ml/min and the column temperature was maintained at ambient and the chromatogram was monitored at a wavelength of 266 nm. The injection volume was 20 μl. Buffer pH 3 was used as a diluent.

**Preparation of solvents and solutions**

**Buffer preparation**

Dissolved 1.02 g of Potassium dihydrogen ortho phosphate in 500 ml of water. Mixed the contents with a clean glass rod to dissolve. Then it was sonicated and vacuum filtered through 0.45 μ Millipore

PVDF (poly vinyl difluride) filter. The pH was adjusted to 3.0 with ortho phosphoric acid.

**Mobile phase proportion**

Buffer (pH 3) and Methanol were mixed in the ratio of 50:50.

**Diluent preparation**

Buffer (pH 3) was used as the diluent.

**Standard stock solution preparation ( 100ppm)**

An accurately weighed quantity of Zidovudine API, 10mg, was transferred into 10ml volumetric flask and then made up to volume with diluent.

**Sample solution preparation**

0.1ml of stock solution was diluted to 10 ml with diluent to prepare 10 ppm.

**Determination of detection wavelength for Zidovudine API**

Appropriate dilutions of the standard drug solutions were prepared for 10 ppm of zidovudine API. 10 ppm solution of Zidovudine was prepared in methanol as a diluent. Solution was scanned using double beam UV VIS spectrophotometer between the range of 200 to 400 nm. λmax of 266 nm was considered for experimental work. The UV spectrum is shown in fig.1.
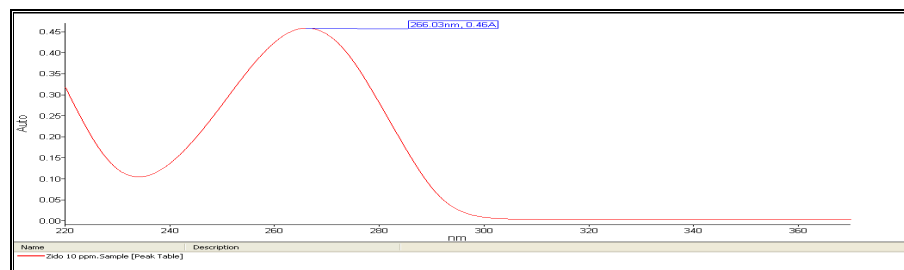


**Fig. 1: UV spectra of Zidovudine for detection wavelength; (λ$_{max}$) = 266 nm**

**Method development by QbD approach**

**Step 1: Define method intent [13-17]**

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 separate and quantify the main compound

**Step 2: Perform experimental design [17-24]**

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 RP-HPLC method (column, pH and mobile phase) is presented. It forms a chromatographic database 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 chromatographic column used no longer be commercially available, or an impurity is no longer relevant. The scoutings of three parameters are shown in table 2.

An experimental design comprised of a standard set of 2 columns, 5 pH values and 4 mobile phase was developed. This led to a total of 40 (2 columns x 5 pH x 4 mobile phase) chromatographic conditions. For each column/pH/Organic modifier combination, a 10 run time was there. In addition, it enabled the creation of a database that

describes the relationship of the compound retention and possible RP-HPLC conditions.

**Table 2: Scouting of three parameters of HPLC**

| Parameters | Description of parameters |
|---|---|
| Column | C8 |
| | C18 |
| pH (buffer) potassium dihydrogen phosphate. pH adjusted with phosphoric acid. | 2 |
| | 3 |
| | 4 |
| | 5 |
| | 6 |
| Mobile phase | ACN:Buffer |
| | ACN:Water |
| | Methanol:Water |
| | Methanol:Buffer |

**Step 3: Evaluate experimental results and select final method conditions [25-29]**

The 40 method conditions were evaluated using the three tiered approach. At the first level, the conditions were evaluated for peaks symmetry, peaks fronting and peaks tailing. This resulted in 20 chromatographic conditions for API. At the second level, these 20 conditions were further evaluated by using more stringent criteria, such as tailing factor should be less then 1.5, etc.

**Step 4: Perform risk assessment with robustness and ruggedness evaluation [29-33]**

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.

**A) Robustness[34]**

To establish the robustness of test method and to demonstrate its reliability for minor changes in chromatographic condition

**B) Ruggedness[35]**

The ruggedness of analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, different instruments, different lots of reagents, different assay, temperatures, different days, different analysts, etc.

**Step 5: Define analytical method performance control strategy**

As a result of robustness and ruggedness studies, the overall method understanding of method performance under various conditions can be improved and an analytical method performance control strategy along with appropriate system suitability criteria can be defined to manage risk and ensure the method delivers the desirable method attributes. If the risk is high and is hard to manage, it is an opportunity for the analyst to go back to the database described in step 2 to find a more appropriate method and to go through the procedure as described to ensure method robustness and ruggedness.

**Analytical method validation[37]**

Validation is documented evidence, which provide a high degree of assurance for specific method. Validation is analytical process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirement for intended analytical application

**Linearity**

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range.

**Linearity solutions preparations**:

1) Level - 50 %( 5 ppm of Zidovudine)
2) Level - 100% (10 ppm of Zidovudine)
3) Level - 150% (15 ppm of Zidovudine**)**

**Precision**

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

**Method precision (Repeatability)**

**Procedure:** Method precision was established by determining six sample preparations under same conditions. Six replicates of sample were prepared at sample concentration by one analyst and analyzed on same day.

**LOD and LOQ**

The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy

Under the stated experimental conditions, It is expressed as the concentration of analyte (e.g. percentage, parts per billion) in the sample. S/N ratio not less than 10

**System suitability**

System suitability was performed and calculated at the start of study of each validation parameter. The values of system suitability results obtained during the entire study were recorded.

**Acceptance criteria**

- Asymmetry of both the analytes peak in standard should not be more than 1.0
- Theoretical plates of both the analytes peak in standard should not be less than 2000
- Relative Standard Deviation for five replicates injections of both the standard preparation should not be more than 2.0%
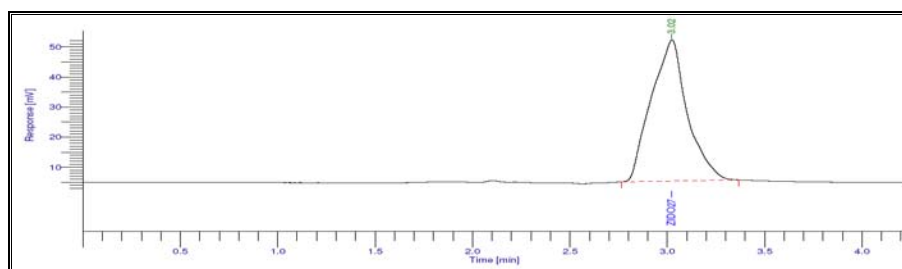
**RESULTS AND DISCUSSION**

**Method development for C8**

Al the trials are shown in table 3 and the chromatogram are shown in fig. 2.

**Table 3: Observation and remarks of method development for C8**

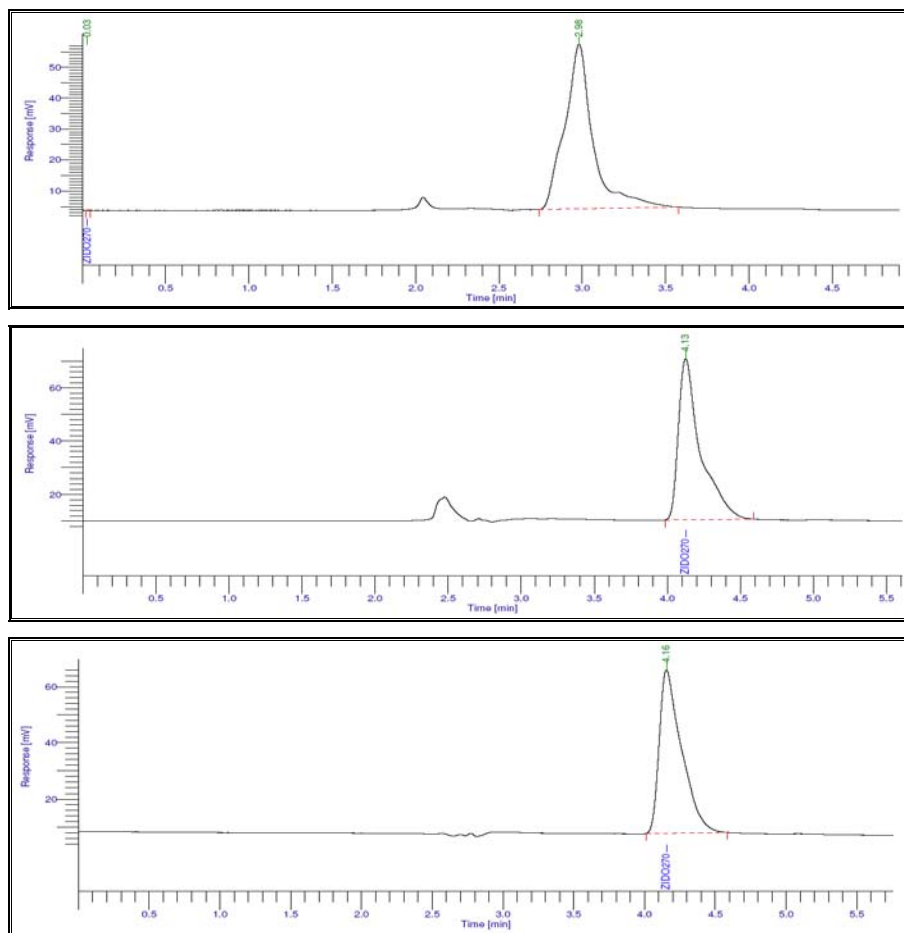| Sr. No. | Trails taken | Observation | Remarks |
|---|---|---|---|
| 1 | ACN: Buffer (pH 3) (50:50%v/v) | Peak was found to be very asymmetrical. | Not Satisfactory |
| 2 | ACN: Water (50:50%v/v) | Peak was found to be very asymmetrical and tailing was obtained. | Not Satisfactory |
| 3 | Methanol: Water (50:50%v/v) | No good Peak Shape | Not Satisfactory |
| 4 | Methanol: Buffer (pH 3) (50:50%v/v) | Sharp peak was obtained but tailing factor was more than 1.5 | Not Satisfactory |

**Fig. 2: Chromatogram of trial 1, trial 2, trial 3 and trial 4 of C8**

**Method development for C18**

Al the trials are shown in table 4 and the chromatogram are shown in fig. 3.

**Table 4: Observation and remarks of method development for C18**

| Sr. No. | Trails Taken | Observation | Remarks |
|---|---|---|---|
| 1 | ACN: Buffer (pH 3) (50:50%v/v) | Peak was found to be little asymmetrical. | Not Satisfactory |
| 2 | ACN: Water (50:50%v/v) | Peak was found to be little asymmetrical and little tailing was obtained | Not Satisfactory |
| 3 | Methanol: Water (50:50%v/v) | Good symmetrical Peak shape and tailing factor is 1.4 | Not Satisfactory |
| 4 | Methanol: Buffer (pH 3) (50:50%v/v) | Good symmetrical Peak shape and tailing factor is 1.34 | Satisfactory |
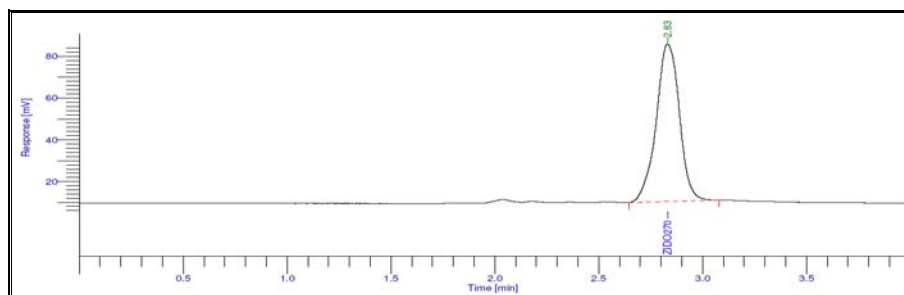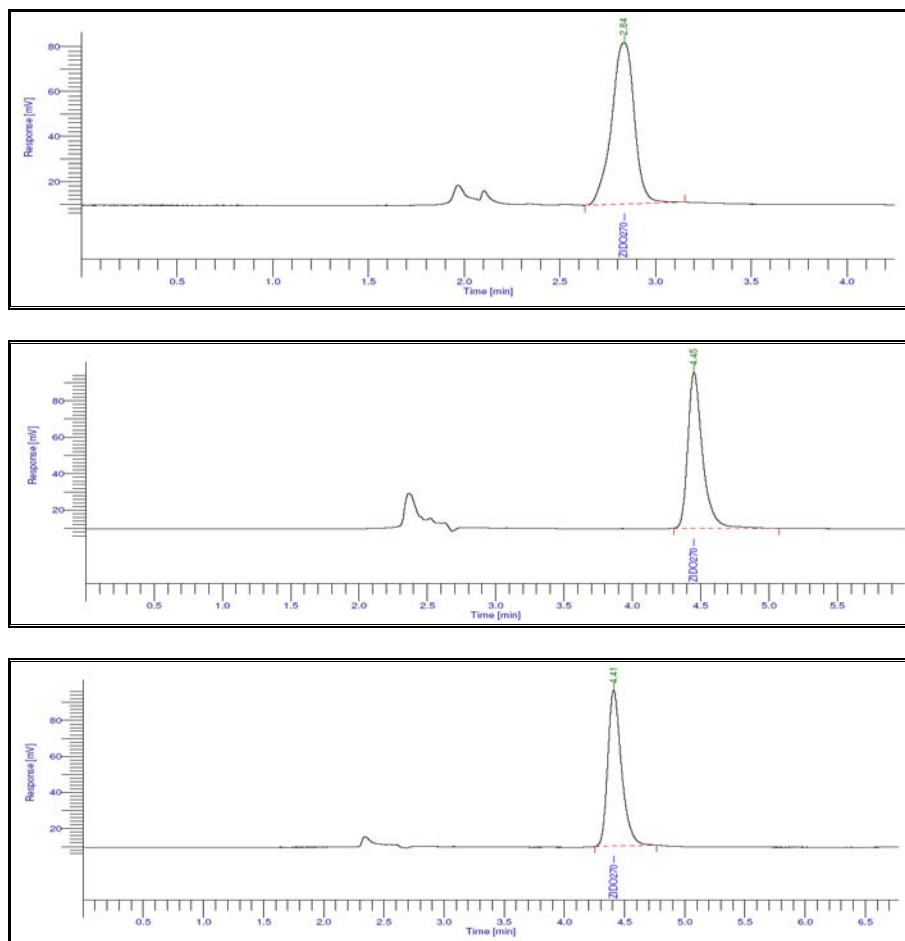
**Fig. 3: Chromatogram of trial 1, trial 2, trial 3 and trial 4 of C18**

**Method development for C18 using different pH conditions**

Al the trials are shown in table 5 and the chromatogram are shown in fig. 4.

**Table 5: Observation and remarks of method development in various pH buffers**

| Sr. No. | Trails Taken | Observation | Remarks |
|---|---|---|---|
| 1 | Methanol: Buffer (pH 2) (50:50%v/v) | Peak was found to be very asymmetrical and splitting of peak was there. | Not Satisfactory |
| 2 | Methanol: Buffer (pH 3) (50:50%v/v) | Peak was found to be very symmetrical | Satisfactory |
| 3 | Methanol: Buffer (pH 4) (50:50%v/v) | Tailing was observed. | Not Satisfactory |
| 4 | Methanol: Buffer (pH 5) (50:50%v/v) | Peak was found to be very asymmetrical and fronting was observed. | Not Satisfactory |
| 5 | Methanol: Buffer (pH 6) (50:50%v/v) | Peak was found to be very asymmetrical and fronting was observed. | Not Satisfactory |
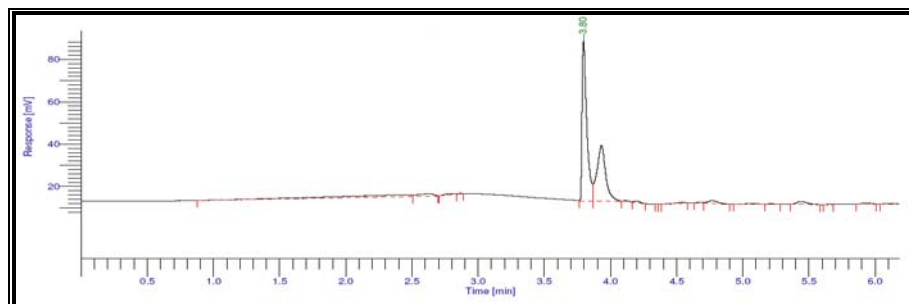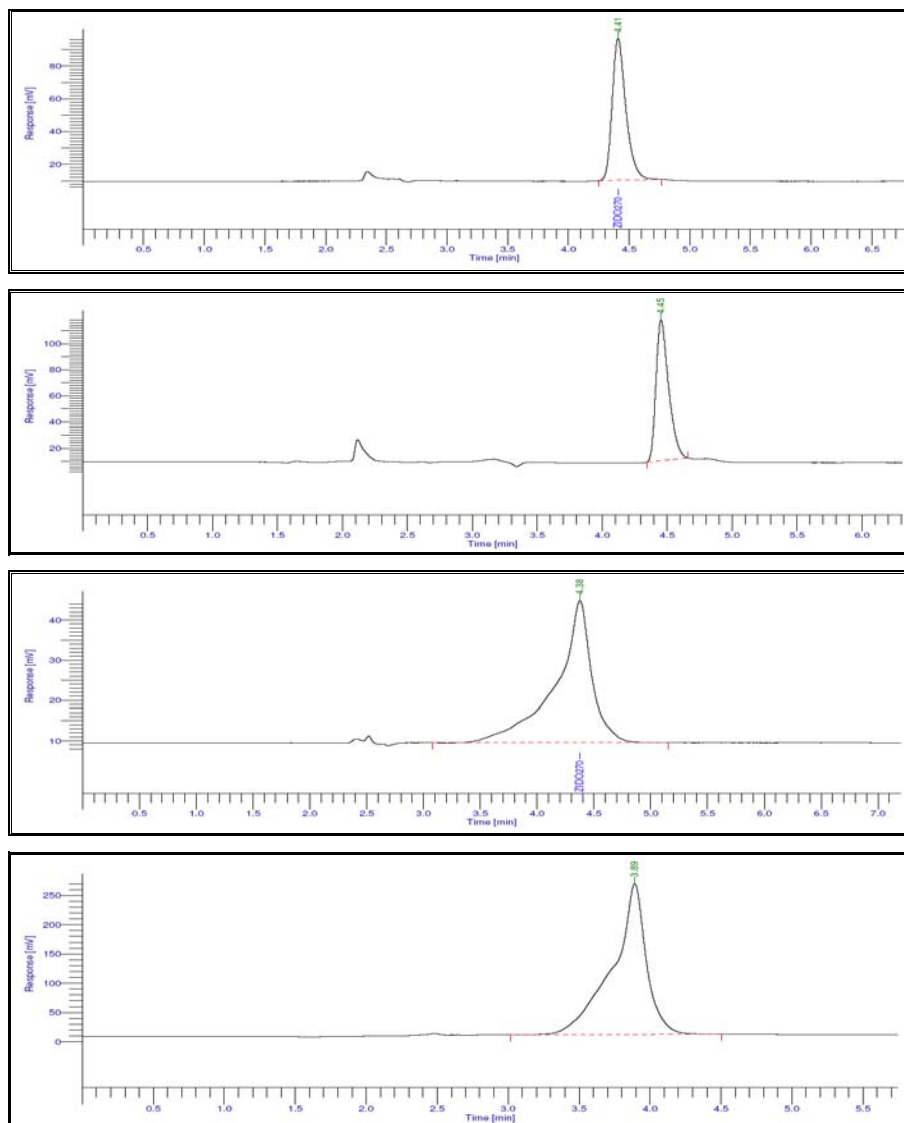
**Fig. 4: Chromatogram of trial 1 of pH 2, trial 2 of pH 3, trial 3 of pH 4, trial 4 of pH 5 and trial 5 of pH 6**

**Final Method Conditions**
Column         : C -18 250mm×4.6mmcolumn
Mobile Phase: Buffer pH 3: methanol (50:50)
Flow         : 1.0 ml/ min
Inj. Volume: 10 µl
Wave length: 266 nm

Oven temp.  : 25°C
Run time     : 10 min
Diluent      : Buffer pH 3
Blank prep.  : Diluent
**Robustness**
The result is shown in table 6.

**Table 6: Results of robustness**

| Robustness | % RSD |
|---|---|
| | **Zidovudine** |
| Methanol: Buffer (pH 3) (48:52%v/v) | 1.023 |
| Methanol: Buffer (pH 3) (52:48%v/v) | 1.253 |
| Wavelength: 264 from 266 | 0.627 |
| Wavelength: 268 from 266 | 0.639 |
| Flow Rate: 0.9 ml/min | 1.133 |
| Flow Rate: 1.1ml/min | 1.127 |
| pH of mobile phase buffer: 2.8 from 3.0 | 1.655 |
| pH  of mobile phase buffer: 3.2 from 3.0 | 1.205 |

**Conclusion:** The study proves the reliability of test method for minor changes in chromatographic condition.

**Ruggedness:** The result is shown in table 7.

**Table 7: Results of ruggedness**

| Day 1, Analyst 1 | | Day 8, Analyst 2 | |
|---|---|---|---|
| Samples | Area | Samples | Area |
| 1 | 659065.52 | 1 | 663876.36 |
| 2 | 688959.06 | 2 | 679659.1 |
| 3 | 686766.00 | 3 | 672828.78 |
| | 678464.37 | | 662132.72 |
| 5 | 673991.87 | 5 | 662111.37 |
| 6 | 694986.45 | 6 | 682737.23 |
| Mean | 680538.86 | Mean | 670556.10 |
| S.D | 12833.799 | S.D | 9202.674 |
| R.S.D | 1.886 | R.S.D | 1.372 |

**Conclusion:** The study proves the reliability of test method for ruggedness in chromatographic condition.
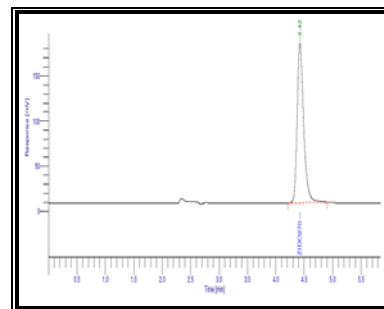
**Linearity**

The linearity results are shown in table 8 and the graphs are shown in fig. 5.

**Table 8: Results of linearity**

| Linearity Conc. | Response (Area) | Mean, Response (Area) |
|---|---|---|
| 5 ppm (50%) | 345994.3 | |
| | 339583.50 | 339470.76 |
| | 332834.43 | |
| 7.5 ppm | 518389.2 | |
| | 518394.5 | 519206.15 |
| | 520834.7 | |
| 10 ppm (100%) | 697618.8 | |
| | 669082.4 | 678941.54 |
| | 670123.4 | |
| 12.5 ppm | 858672.8 | |
| | 857234.5 | 858676.92 |
| | 860123.4 | |
| 15 ppm (150%) | 1018821 | |
| | 1017093 | 1018412.3 |
| | 1019323 | |
| Slope | | 68250 |
| Regression coefficient | | 0.9998 |
| Regression equation | | y = 68250x + 1428.6 |
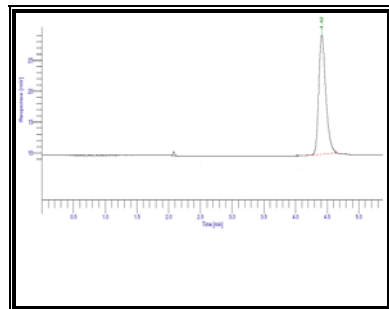


**50 % (5 ppm)**



**(c) 150% (15 ppm)**
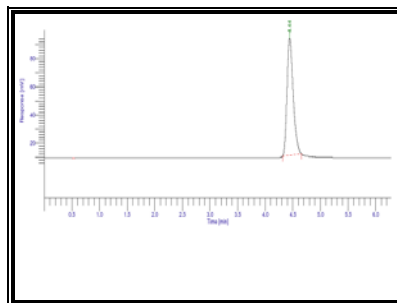


**(a)  100 % (10 ppm)**
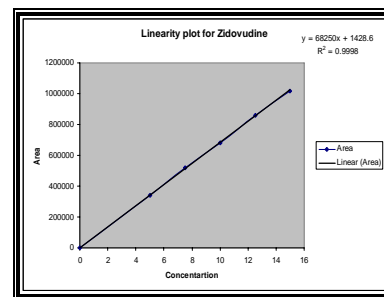


**(d) Linearity plot for zidovudine**

**Fig. 5:  (a): 50 % (5 ppm), (b): 100% (10 ppm), (c): 150 % (15 ppm), (d): Linearity plot for zidovudine**

**CONCLUSION**

From the study of concentration range, the linear response for the analyte exist can be established.
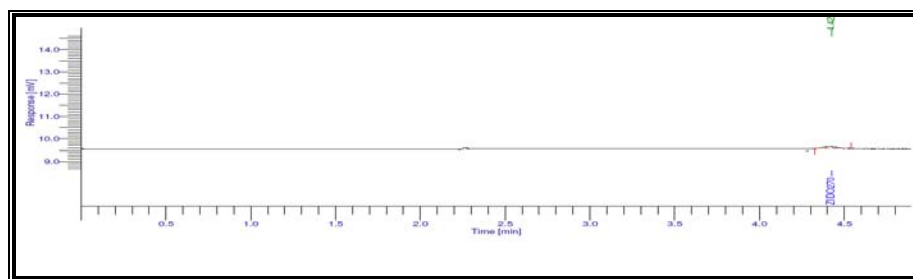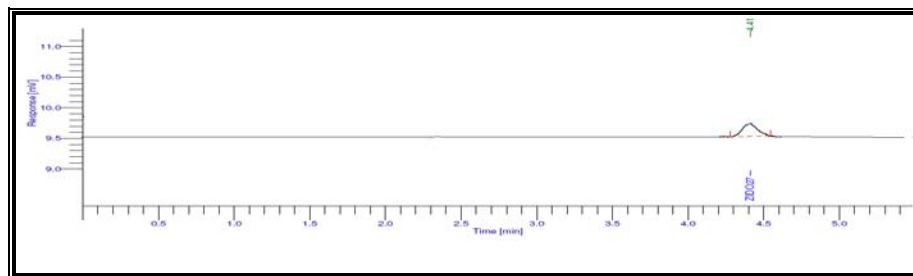
**Precision**

The RSD of six replicate injection of zidovudine is 0.79% and thus it is within the accepted criteria. (RSD NMT 1.0%).

**LOD and LOQ**

They are mentioned in table 9 and the chromatograms are shown in fig.6a and fig.6b

**Table 9: Results of LOD and LOQ**

| Sr. No. | LOD | | LOQ | |
|---|---|---|---|---|
| | Conc. (ng/ml) | S/N ratio | Conc. (ng/ml) | S/N ratio |
| 1 | 4.2 | 2.95 | 12.0 | 9.824 |



**Fig. 6 (a): Chromatogram of Zidovudine at LOD level**



**Fig. 6(b) : Chromatogram of Zidovudine at LOQ level**

**CONCLUSION**

The results obtained were within the acceptance criteria.

**System suitability parameters**

The mean values of system suitability parameters are shown in table 10.

**Table 10: Results of mean values of System Suitability parameters**

| System Suitability parameter | Observation Zidovudine | Acceptance criteria |
|---|---|---|
| Tailing factor of analyte peak | 1.3 | Should NMT 2 |
| Theoretical plate of analyte peak | 7445 | Should NLT 2000 |
| % RSD of 6 injection | 0.79 | Should NMT 1 |

**CONCLUSION**

A reversed phase HPLC method development approach using QbD principles has been described. First, the method goals are clarified based on the process understanding. The experimental design describes the scouting of the key HPLC method components including column, pH, and mobile phasse. Their interrelationships are studied and the preliminary optimized conditions are obtained for each combination of column, pH and mobile phase. Here a better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the methods to meet their intended purposes is done. Moreover, this approach provides an in-depth knowledge and enables the creation of a chromatographic database that can be utilized to provide alternative method conditions at a future time should changes to the method be required. Furthermore, the method development is not considered finished until a thorough risk assessment and all the necessary robustness and ruggedness studies are carried out. All the validated parameters were found within acceptance criteria. The validated method is specific, linear, precise, accurate, robust and rugged for determination Based on the knowledge of method obtained through the method development and the results of risk assessment along with robustness and ruggedness studies, detailed analytical method performance control strategy can be defined to manage the risk. This approach has been successfully used in the laboratory to develop HPLC method for zidovudine

API. Moreover, QbD approach has even the potential for simultaneous development of multiple methods including impurity methods, assay method, dissolution method, cleaning validation method, etc and thus it should be implemented.

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