IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2022 Vol.:25, Issue:4

A Novel RP-HPLC Method Development and Validation for Simultaneous Estimation of Emtricitabine and Tenofovir Disproxil Fumarate in Active Pharmaceutical Ingredients and Marketed Combined Tablet Dosage Forms

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

G. Shireesha, *A. Santhosh, Sareesh Kankanala, P. Sunil Kumar Chaitanya

Department of Pharmaceutical Analysis, St Pauls College of Pharmacy, Turkayamjal, Hyderabad, Telangana-501510, India

Submitted: 25 October 2022
Accepted: 31 October 2022
Published: 30 November 2022

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Emtricitabine, Tenofovir Disoproxil Fumarate, RP-HPLC, ICH Guidelines.

ABSTRACT

A rapid, precise, accurate, specific and simple RP-HPLC method was developed for the simultaneous estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in bulk and its combined pharmaceutical dosage form. A High performance liquid chromatograph WATERS, software: Empower 2, 2695 separation module, 996 PDA detector, using Phenomenex Luna C18 (4.6mm x 250mm, 5µm, Make: Waters) or equivalent column, with mobile phase composition of Acetonitrile: Phosphate Buffer (pH-6.8) [20:80 % (v/v)] was used. The flow rate of 1.0 ml min-1 and effluent was detected at 262 nm. The retention time of Emtricitabine and Tenofovir Disoproxil Fumarate was found to be 2.242 min and 3.678 minutes respectively. Linearity was observed over concentration range of 30-70µg ml-1 for Emtricitabine and 60-140µg ml-1 for Tenofovir Disoproxil Fumarate respectively. The accuracy of the proposed method was determined by recovery studies and the Alprazolam was found to be 100.41% and Sertraline was found to be 99.58% respectively. The proposed method is applicable to stability studies and routine analysis of Emtricitabine and Tenofovir Disoproxil Fumarate in bulk and pharmaceutical formulations. The proposed method was validated for various ICH parameters like linearity, limit of detection, limits of quantification, accuracy, precision, range and specificity.

INTRODUCTION

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. The current good manufacturing practice (CGMP) and food drug administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Development of a method of analysis is usually based on prior art (or) existing literature, using the same (or) quite similar instrumentation. It is rare today that an HPLC-based method is developed that does not in same way relate (or) compare to existing, literature based approaches. Today HPLC (high performance liquid chromatography) is the method of choice used by the pharmaceutical industry to assay the intact drug and degradation products. The appropriate selection and chromatographic conditions ensure that the HPLC method will have the desired specificity. UV spectroscopy is also a simple analytical tool widely used for routine assay of drugs. Hence for the assay of the selected drugs HPLC and UV spectroscopy has been chosen for these proposed methods.[1-5]

The developed chromatographic methods further validated as per ICH or USFDA guidelines for all the critical parameters. To access the precision and to evaluate the results of analysis the analyst must use statistical methods. These methods include confidence limit, regression analysis to establish calibration curves. In each analysis the critical response parameters must be optimized and recognized if possible.

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches like chemistry, physics and microbiology etc. Pharmaceutical analytical techniques are applied mainly in two areas, quantitative analysis and qualitative analysis, although there are several other applications.

Drugs and pharmaceuticals are chemicals or like substances, which or of organic inorganic or other origin. Whatever may be the origin, we some property of the medicinal agent to measure them quantitatively or qualitatively.[5-10]

In recent years, several analytical techniques have been evolved that combine two or more methods into one called “hyphenated” technique e.g. GC/MS, LC/MS etc. The complete analysis of a substance consists of four main steps.

The concept of analytical chemistry lies in the simple, precise and accurate measurements. These determinations require highly sophisticated instruments and methods like mass spectroscopy, gas chromatography, high performance thin layer chromatography, high performance liquid chromatography etc. The HPLC method is sensitive, accurate, precise and desirable for routine estimation of drugs in formulations.

Thereby it is advantageous than volumetric methods. Many HPLC methods has been developed and validated for the quantitative determination of various marketed drugs.

Analytical method development and validation places an important role in drug discovery and manufacture of pharmaceuticals. These methods are used to ensure the identity, purity, potency and performance of drug products majority of analytical development effort goes into validating a stability indicating method. So it is a quantitative analytical method based on the structure and chemical properties of each active ingredient of the drug formulation.

Most of the drugs can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision, reproducibility, ease of automation and eliminates tedious extraction and isolation procedures.

On the literature survey, it was found that most of the analytical method available for the above mentioned drug is applicable for quantification in plasma samples, the most widely used method being liquid chromatography-mass chromatography. So it is felt that there is a need to develop accurate, precise analytical methods for the estimation of the drug in solid dosage formulation. [10-16]

MATERIALS AND METHODS

Emtricitabine from Sura labs, Tenofovir disproxil fumarate from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Anhydrous dihydrogen phosphate from Finar chemicals, Phosphate Buffer from Finar chemicals, Citric Acid from Finar chemicals.

HPLC METHOD DEVELOPMENT:

Mobile Phase Optimization:

Initially the mobile phase tried was Acetonitrile: Water and Acetonitrile: Sodium dihydrogen phosphate buffer with varying proportions.

Finally, the mobile phase was optimized to Acetonitrile with Sodium dihydrogen phosphate buffer (pH 6.8), in proportion 20:80 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, X- bridge column, Xterra, and C8 column. Phenomenex Luna C18 (4.6mm x 250mm, 5µm, Make: Waters) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 Model.
Temperature	:	Ambient
Column	:	Phenomenex Luna C18 (4.6mm x 250mm, 5µm, Make: Waters) or equivalent
Buffer	:	1.1998gm of Sodium dihydrogen phosphate in 1000ml HPLC water pH (6.8) adjusted with ortho phosphoric acid.
pH	:	6.8
Mobile phase	:	80% buffer 20% Acetonitrile
Flow rate	:	1 ml per min
Wavelength	:	262 nm
Injection volume	:	10 µl
Run time	:	6min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Phosphate buffer:

Accurately 1.1998 gm of Sodium dihydrogen phosphate was taken in a 1000 ml volumetric flask, dissolved in 150 mL of HPLC water and adjusted to pH 6.8 with Orthophosphoric acid diluted to 1000ml with HPLC water and filtered by using 0.45µ filter paper and sonicated.

Preparation of mobile phase:

Accurately measured 800 ml (80%) of above buffer and 200 ml of HPLC grade acetonitrile (20%) were mixed and degassed in a digital ultra sonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

(Optimized Chromatographic Condition)

Mobile phase:	Phosphate buffer (0.01M) pH 6.8: Acetonitrile (80:20%v/v)
Column:	Phenomenex Luna C18 (4.6mm x 250mm, 5µm Particle size Make: Waters) or equivalent
Flow rate:	1 ml/min
Wavelength:	262 nm
Column temp:	Ambient
Sample Temp:	Ambient
Injection Volume:	10 µl

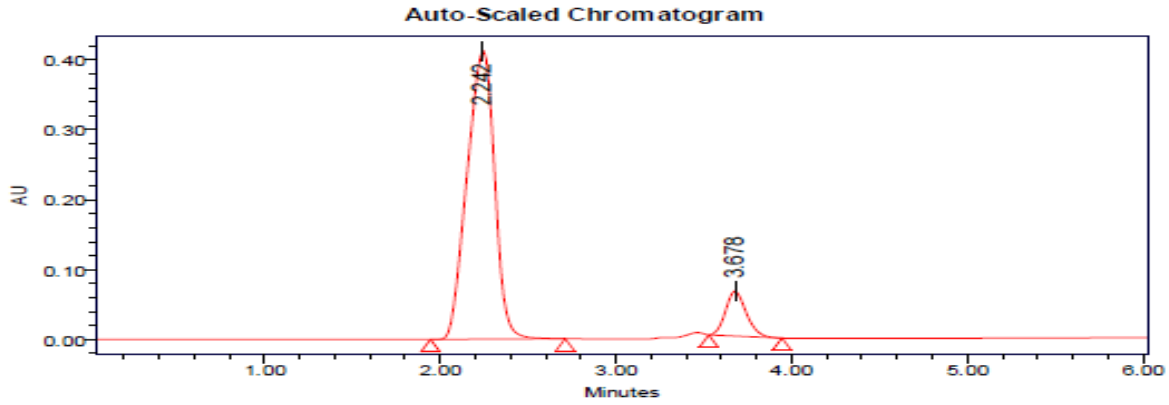

Fig. No. 1: Optimized Chromatographic Condition (Emtricitabine + Tenofovir disproxil fumarate)

Table No. 1: Results of Optimized Chromatographic Condition (Emtricitabine + Tenofovir disproxil fumarate)

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Emtricitabine	2.242	4256351	565842		0.68	6584
2	Tenofovir disproxil fumarate	3.678	265284	285441	3.6	1.47	4857

From the above chromatogram it was observed that the Emtricitabine and Tenofovir disproxil fumarate peaks are well separated.

Retention time of Emtricitabine– 2.242 min

Retention time of Tenofovir disproxil fumarate – 3.678 min

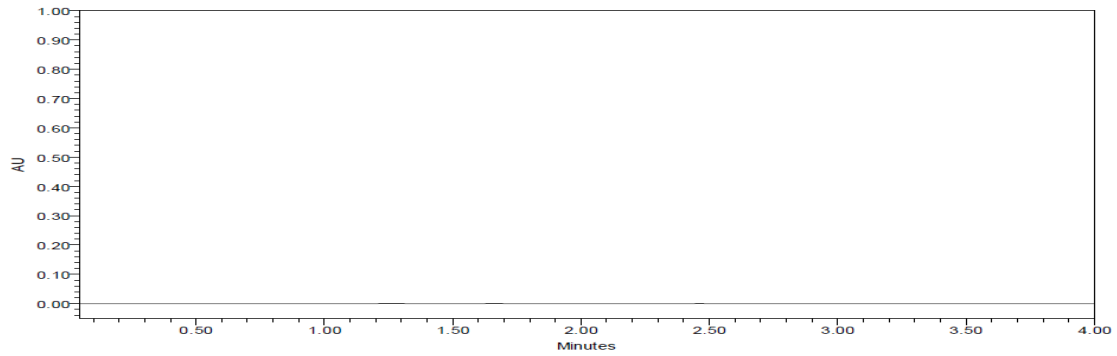

Fig. No. 2: Chromatogram for Blank Solution

From the above chromatogram it was observed that there are no interferences.

SYSTEM SUITABILITY: System suitability: A Standard solution was prepared by using emtricitabine and tenofovir disproxil fumarate working standards as per test method and was injected in replicates for five times into the HPLC system. The system suitability parameters like theoretical plates, tailing factor, resolution were evaluated from standard chromatograms. The results were given in table-2.

Table No. 2: Results of system suitability parameters for Emtricitabine and Tenofovir disproxil fumarate

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Emtricitabine	2.242	4263524	545145		0.85	7568
2	Tenofovir disproxil fumarate	3.679	267412	27582	3.9	1.26	4214

All the system suitability parameters for developed method were within the limits.

VALIDATION PARAMETERS:

$$\% \text{ ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Emtricitabine and Tenofovir disproxil fumarate in pharmaceutical dosage form was found to be 100.016%.

Precision

Method precision: Sample solutions were prepared as per the test procedure and six injections were given in replicates.

Intermediate Precision: To evaluate the intermediate precision, studies were performed on different days by maintaining same conditions. The standard solution was injected for six times in replicates. The peak areas for all six injections were recorded and the %RSD for the same was calculated and reported. The procedure under similar conditions was repeated on day two. The results were given in tables 3 to 5.

Table No. 3: Results of method precision for Emtricitabine &Tenofovir disproxil fumarate

S. NO	Emtricitabine Peak areas	Tenofovir disproxil fumarate Peak areas
1	4263582	266521
2	4265851	225542
3	4285422	225542
4	4225594	265648
5	4275845	265845
% RSD	0.108	0.85

INTERMEDIATE PRECESSION (ruggedness)

Table No. 4: Results of Intermediate precision Day 1 for Emtricitabine + Tenofovir disproxil fumarate

Injection	Peak Areas of Emtricitabine	% Assay	Peak Areas of Tenofovir disproxil fumarate	% Assay
1	4254784	100.37	266521	99.56
2	4225947	100.47	225542	99.87
3	4289354	100.42	225542	99.65
4	4289354	100.22	265648	99.67
5	4225594	100.42	265845	99.76
6	4275845	100.30		
% RSD	0.106		0.88	

The method was precise and rugged as the % RSD of peak areas is less than 2.

ACCURACY: Accuracy of the method was evaluated by recovery studies. Three target concentrations 50%, 100%, 150% were prepared with respect to target assay and injected into HPLC system in triplicates. At each spike level the mean recovery values are between 98 to 102 % which were in agreement with the acceptance criteria. The recovery values indicate the method is accurate. The results are observed in table V & VI.

Table No. 5: Accuracy (recovery) data for Emtricitabine

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3372980	40	39.893	99.732%	100.41%
100%	4285059	50	50.617	101.234%	
150%	5085059	60	60.163	100.271%	

Table No. 6: Accuracy (recovery) data for Tenofovir disproxil fumarate

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	209948	40	39.612	99.03%	99.58%
100%	262097	50	49.538	99.076%	
150%	318874	60	60.383	100.638%	

The mean recovery for emtricitabine and Tenofovir disproxil fumarate was found to be 100.41% and 99.58%.

LINEARITY: Linearity of the method was evaluated by injecting various concentrations of both the drugs into HPLC system. A graph was plotted with peak area versus concentration and the correlation coefficient was calculated. The r^2 values of both the drugs were found to 0.999 which were within the limits. The r^2 values confirmed the method was linear and the results were shown in table 8&9 and figures 7&8.

Table No. 7: Results for Linearity for Emtricitabine

S. No.	Linearity Level	Concentration	Area
1	I	30 ppm	158547
2	II	40 ppm	215475
3	III	50 ppm	265284
4	IV	60 ppm	319866
5	V	70 ppm	365214
Correlation Coefficient			0.999

Table No. 8: Results for Linearity for Tenofovir disproxil fumarate

S. No.	Linearity Level	Concentration	Area
1	I	60ppm	2544547
2	II	80ppm	3358542
3	III	100ppm	4231546
4	IV	120ppm	5127547
5	V	140ppm	5874451
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.99.

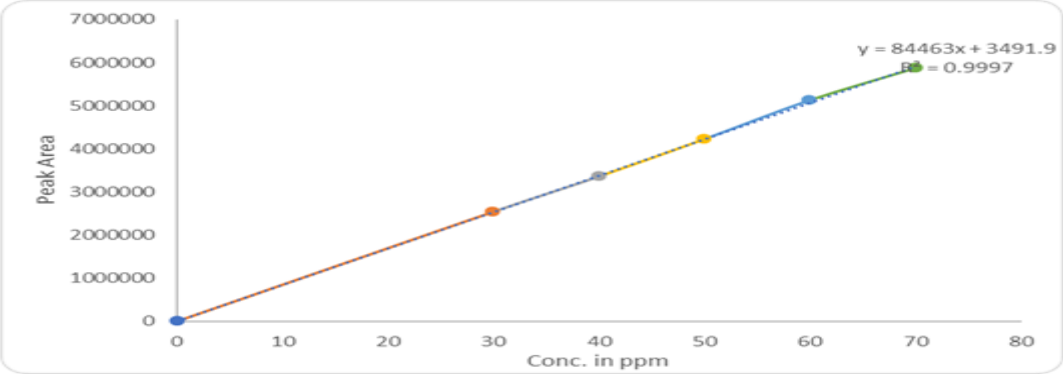

Fig. No. 3: Calibration graph for Emtricitabineat 262 nm

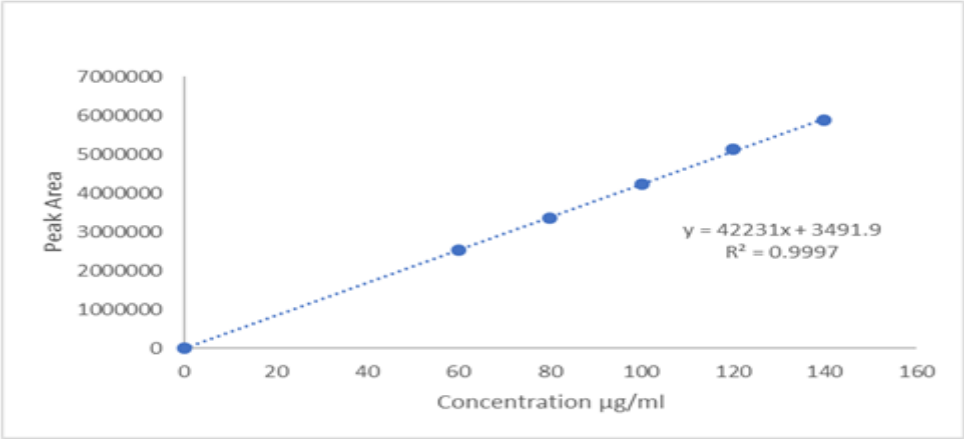

Fig. No. 4: Calibration graph for Tenofovir disproxil fumarate at 262 nm

Table No. 9: Analytical performance parameters of Emtricitabine and Tenofovir disproxil fumarate

Parameters	**Emtricitabine**	**Tenofovir disproxil fumarate**
Slope (m)	84463	42231
Intercept (c)	3491	3491
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria:

Correlation coefficient (R^2) for both the analytes was found to be should not be less than 0.999.

- The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearity was established in the range of 10% to 50% of Emtricitabine and 1% to 5% of Tenofovir disproxil fumarate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

RESULT:

Emtricitabine:

0.8µg/ml

Tenofovir disproxil fumarate:

0.7µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ=10\times\sigma/S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

RESULT:

Emtricitabine:

2.4µg/ml

Tenofovir disproxil fumarate:

2.19µg/ml

ROBUSTNESS:

System suitability results for Emtricitabine: A study was carried out with variation in flow rate to evaluate the robustness of the method. The standard solutions were injected in the selected robust conditions and the system suitability parameters like theoretical plates, tailing factor and resolution were observed. The results showed that the theoretical plate count was more than 2000, tailing factor was less than 2 and resolution was found more than 2. The results of the study indicated that the method was robust and the results were shown in table 10 &11.

Table No. 10: System suitability results for Emtricitabine

Sr. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	U SP Tailing
1.	0.8	6686	0.69
2.	1.0	6584	0.68
3.	1.2	6785	0.67

***** Results for actual flow (1.0 ml/min) have been considered from Assay standard.

Table No. 11: System suitability results for Tenofovir disproxil fumarate

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1.	0.8	4986	1.49
2.	1.0	4857	1.47
3.	1.2	4998	1.53

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

Variation of mobile phase organic composition:

Table No. 12: System suitability results for Emtricitabine

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6087	0.59
2	*Actual	6584	0.68
3	10% more	6989	0.57

Table No. 13: System suitability results for Tenofovir disproxil fumarate

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4169	1.39
2	*Actual	4857	1.47
3	10% more	4468	1.38

***** Results for actual mobile phase have been considered from Assay standard.

CONCLUSION

A new method was established for simultaneous estimation of Emtricitabine and Tenofovir disoproxil fumarate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Emtricitabine and Tenofovir disoproxil fumarate by using

Phenomenex Luna C18 (4.6mm x 250mm, 5µm, Make: Waters) or equivalent, flow rate was 1ml/min, mobile phase ratio was (20:80 v/v) Acetonitrile: Phosphate buffer pH 6.8 (pH was adjusted with orthophosphoric acid), detection wave length was 262nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.242mins and 3.678mins. The % purity of Emtricitabine and Tenofovir disoproxil fumarate was found to be 99.85% and 100.14% respectively. The system suitability parameters for Emtricitabine and Tenofovir disoproxil fumarate such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study on Emtricitabine and Tenofovir disoproxil fumarate was found in concentration range of 30µg-70µg and 60µg-140µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.41% and 99.83%, % RSD for repeatability was 0.207 and 0.534. The precision study was precise, robust, and repeatable. LOD value was 0.8 and 0.7, and LOQ value was 2.4 and 2.19 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Emtricitabine and Tenofovir disoproxil fumarate in API and Pharmaceutical dosage form.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, St Pauls College of Pharmacy, Turkayamjal for extending support to carry out the research work.

REFERENCES

1. Dr. Kealey and P. J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A. Braith Wait and F. J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla C. G., A new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. Introduction to Column. (Online), URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf.
9. Detectors used in HPLC (online), URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC.
10. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.

11. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.
12. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
13. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
14. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
15. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
16. Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.