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NEW HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF EMTRICITABINE IN MARKETED FORMULATION

Prabhat Tiwari, Dr. Alok Pal Jain, Vivek Shrivastava
RKDF College of Pharmacy, Bhopal, M.P.

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Abstract:
A novel stability-indicating RP-HPLC method has been developed and validated for quantitative analysis of Emtricitabine in the bulk drug and in a pharmaceutical dosage form. An isocratic separation of EMT was achieved on Thermo C18 column (4.6 x 250mm, 5 µ particle size) as the stationary phase with a flow rate of 1 ml/min and using a UV detector to monitor the eluate at 254 nm. The mobile phase consisted of Acetonitrile: methanol (50:50v/v) enabled separation of the drug from its degradation products. The method was validated for linearity, accuracy (recovery), precision, specificity, and robustness. Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curve was linear over the concentration range of 5-25µg/ml and correlation coefficients were found to be 0.999 for Emtricitabine respectively. Recovery studies were carried out by applying the method to drug sample to which known amount of Emtricitabine at three concentration levels of 80, 100% and120 % were added. At each level %recovery was determined, which are in the range of 98.67±0.406- 99.00±0.030%. The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done by measuring the absorbance for six times. The % RSD value was found to be 0.767, 1.224, 1.870 for repeatability, day to day and analyst to analyst respectively indicating that the method is precise. The forced degradation study prove the stability indicating power of the method and therefore, the validated method may be useful for routine analysis of Emtricitabine as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

Keywords: *RP-HPLC, Emtricitabine, Forced degradation, Method validation, repeatability*

Corresponding author:
Prabhat Tiwari,
RKDF College of Pharmacy,
Bhopal, M.P.

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INTRODUCTION:

Analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency and performance of drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physiochemical properties (pKa, logP, solubility) and determining which mode of detection would be suitable for analysis. The majority of the analytical development effort goes into validating a stability indicating HPLC method. The goal of the HPLC method is to try and separate quantify the main active drug, any reaction impurities, all available synthetic intermediates and degradants.

Analysis can be divided in to two classes, i.e. Qualitative analysis and Quantitative analysis. Qualitative analysis gives an indication of the identity of the chemical species in the sample. Quantitative analysis estimates, how much quantity is present in a mixture. Modern analytical chemistry is functioning by instrumental analysis. Separation of components in a mixture is based on their interaction between a stationary and a mobile phase. These interaction differences are achieved based on the properties such as polarity, electric charge (for ionic compounds), pH, functional groups and size of the molecule[5-6].

Different types of quantitative analytical techniques are there, for eg: HPLC, Gas chromatography, TLC, Ion chromatography and Column chromatography, UV/Visible spectroscopy, FT-IR, LC-MS, GC-MS, MASS and NMR.

There are many steps involve in method development which are:

- Physicochemical properties of drug
- Set up HPLC conditions
- Sample preparation
- Method optimization
- Validation of developed method

High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, or ion exchange processes, depending upon the type of stationary phase used[7-8].

HPLC Method Development

Most of the drugs in multicomponent dosage forms can be analyzed by HPLC method for the reason that of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tiresome extraction and isolation procedures. HPLC method development is not very difficult when literature reference for the same or similar compounds to be analyzed can be found.

- Five stages are to be taken into consideration when starting new HPLC method.
- Instrumentation
- Determination of molecular characteristics of sample
- Selection of column
- Selection of mobile phase
- Selection of detector

HPLC Instrumentation [9]

Liquid chromatography (LC) is a physical separation procedure conducted in the liquid phase. A sample is separated into its constituent components (or analytes) by distributing among the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). HPLC is a modern form of LC that uses small- particle columns through which the mobile phase is pumped at high pressure. (Shown in fig. 1)

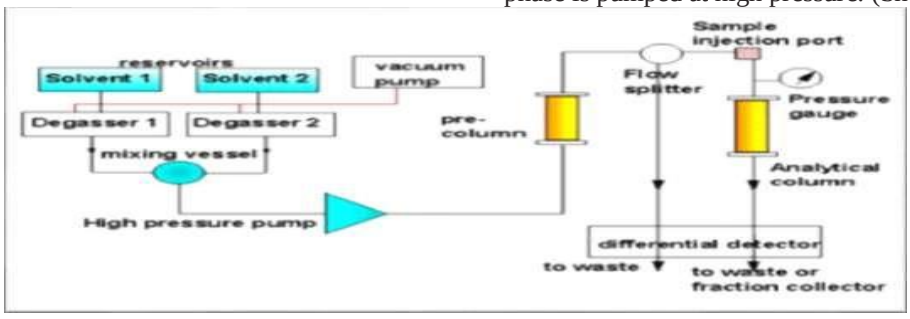

Figure 1: HPLC Instrumentation.

Separation mechanism

Compounds are separated since the molecules travel at different rates in the column. Due to different interaction between stationary phase and different sample, the molecules move at different rate, therefore separation can be done as shown in fig. 2.

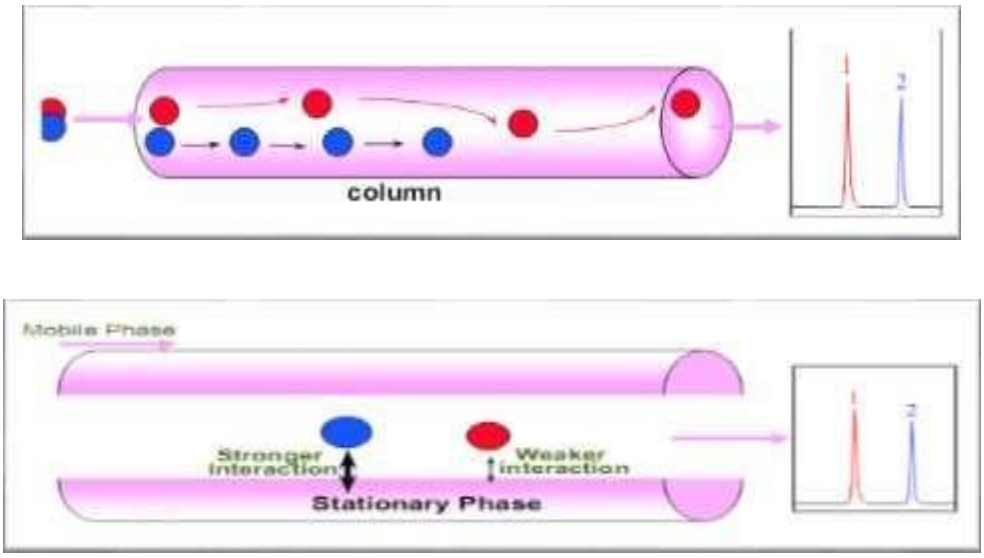

Figure 2: HPLC Separation mechanisms

Analytical Method Validation

The process of establishing documented evidence which provides a high degree of assurance that the method does what it is intended to do.

Positioning of validation in method development process

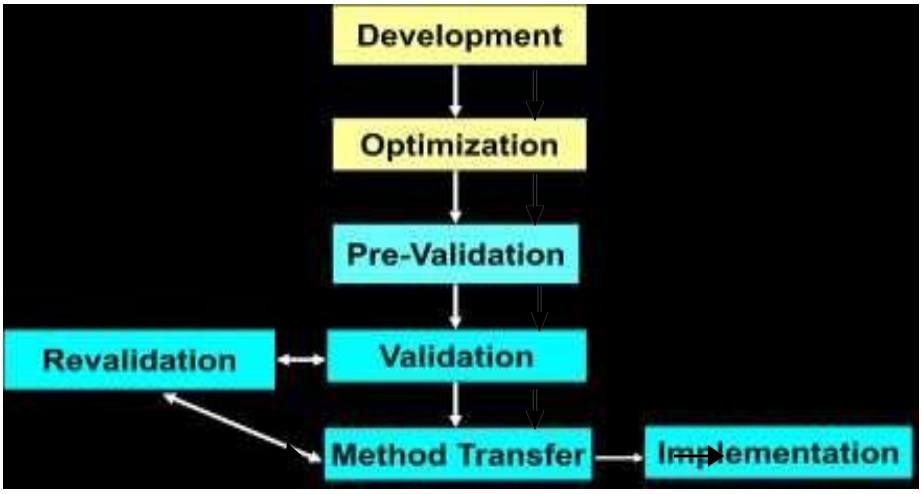

Figure 3: Positioning of validation in method development process.

Table No. 1: Data element required for assay validation (USP)

Performance Parameter	Category I Bulk/Active	Category II (Impurities)		Category III (Performance)	Category IV (Identification)
		Quantitative	Limit tests		
Accuracy	Yes	Yes	□	□	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	□	Yes
Detection limit	No	No	□	□	No
Quantification limit	No	Yes	No	□	No
Linearity	Yes	Yes	No	□	No
Range	Yes	Yes	□	□	No

*May be required, depending on the nature of the specific test.

Stability Studies

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to sustain its identity, strength, quality, and purity all over the retest or expiration dating periods[14].

Physical, chemical, and microbiological data are generated as a function of time and storage conditions (e.g., temperature and relative humidity [RH]). Stability testing provides confirmation that the quality of a drug substance or drug product under the influence of various environmental factors changes with time[15].

Stability plays an important role in the drug development process. It explains several factors that influence the expiration dating of drug products, including the chemical and physical stability during the pre-clinical formulation stages, process development, packaging development, and post-marketing life[16]. (as shown in fig. 8) Stability testing allows the establishment of suggested storage conditions, retest periods, and eventually product shelf-life and expiry dating. In pharmaceutical field stability studies finds an application in the following areas of drug development program [17].

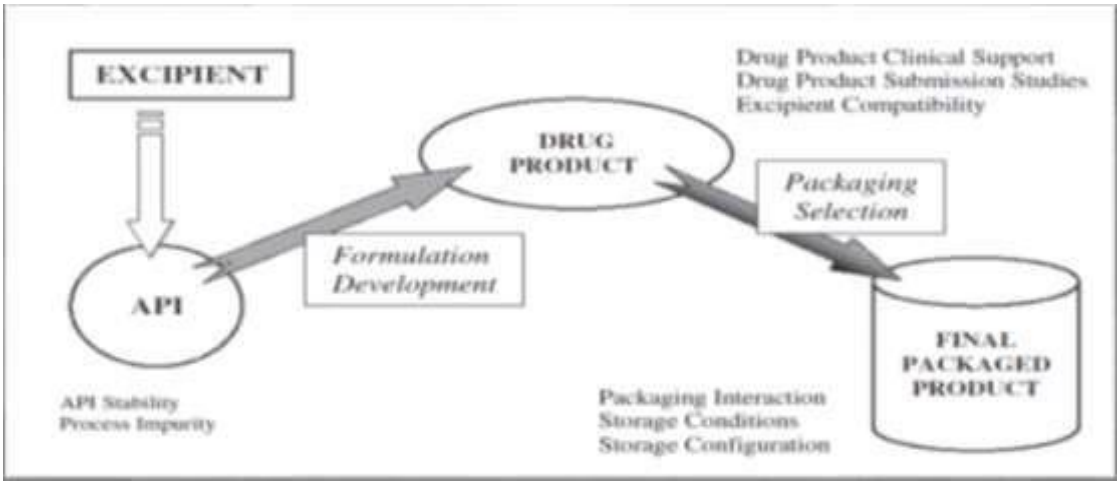

Figure 4 : Stability studies to support development of new drug product

Conducting forced degradation studies

The FDA and International Conference on Harmonization (ICH) guidance provides very little information about strategies and principles for conducting forced degradation studies, together with problems of poorly soluble drugs and exceptionally stable compounds. In particular, the issue of how much stress is adequate in stress testing is not addressed specifically. Overstressing a molecule can lead to degradation profiles that are not representative of real storage conditions and perhaps not applicable to method development. Therefore, stress-testing conditions should be realistic and not excessive. In this regard, it is the amount of stress that is important and not necessarily the extent of degradation. Indeed, some compounds may not degrade significantly after significant exposure to stress conditions [18].

Forced degradation studies should be conducted whenever a stability-indicating method is mandatory. Studies may need to be repeated as methods, processes, or formulations change. Alternatively, methods can be developed with a mixture of the known degradation products, if available[19].

Forced degradation studies should be performed on each unique formulation before formal stability studies initiate. Sufficient exposure of a drug substance or drug product is achieved when the drug substance has degraded ~10% from its preliminary amount and after an exposure in excess of the energy provided by accelerated storage (e.g., 40 ^{0}C for 6 months), whichever comes first. Application of this rule of thumb may end result with no degradation in some cases. The goal is to generate a degradation profile that mimics what would be observed in formal stability studies under ICH conditions[20-21].

Light storage should be in adequate excess of ICH light conditions. The guidance states that solution phase degradation studies of a drug substance can be accepted in solution or in suspension[22].

The use of inert organic co-solvents may be indicated in cases in which drug substances are extremely insoluble but recognize the potential of any organic co- solvent to react with the drug substance under a given set of stress conditions. For drug products, non-drug substance related peaks should be illustrious from drug substance related compounds[23].

This process can be accomplished through comparative analysis of stressed samples of drug substance alone, of drug substance plus excipients, and of excipients alone. Stressing drug substance and/or excipients blends instead of final dosage forms may be enough for the determination of degradation pathways of a drug product. However, there may be significant differences in degradation profiles observed between blends and actual drug products. Consideration also should be given to the prospect of a reaction between the drug substance and components of a film-coating or capsule shell.[24-25].

MATERIALS AND METHODS:

Characterization and identification of Emtricitabine

Physiochemical characteristics

Description- Solid, white to slightly yellow powder.

Solubility- Solubility of Emtricitabine was established by I.P. method.

Identification

FTIR spectrum- IR absorption spectra of Emtricitabine were obtained by KBr pellet method.

Chemicals and solvents

Table No. 1: Chemicals and Solvents Used

S. No.	Chemicals	Manufacturer
1	Emtricitabine	Gift sample, Aurobindo Pharma Limited
2	Methanol (AR Grade)	Merck Ltd., India
3	Acetonitrile (HPLC)	Merck Ltd., India
4	Methanol (HPLC)	Merck Ltd., India
5	Water (HPLC)	Merck Ltd., India

Instruments

- A Labindia (3000 plus) spectrophotometer with 1cm quartzcells.
- The HPLC system consisted of a waters pump, a U.V. Visible detector, a Thermo C18 (250 X 4.60 mm), 5µm column, and data acesoftware.

Marketed formulation
Emtriva 200 mg hard capsules

Determination of solubility
Solubility of Emtricitabine was performed in different solvents.

Analytical method development by HPLC: -
Mobile Phase Selection
Initially to estimate Emtricitabine number of mobile phase in different ratio were tried.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was acetonitrile: Methanol. The mobile phase was filtered through 0.45 filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0ml/min.

Selection of wavelength
10 mg of Emtricitabine was weighed accurately and transferred to a 100ml volumetric flask, and the volume was adjusted to the mark with the mobile phase. From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at Lambda max 254.0 nm.

Selection of Separation Variable
Standard drug solution of Emtricitabine was prepared in different mobile phase and chromatograph was recorded by using different column (5 and 10 µm) at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment.

System Suitability Parameters
Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Emtricitabine10 g/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Preparation of Standard Stock Solution
10mg of Emtricitabine was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of Working Standard Solution
From stock solutions of Emtricitabine, 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of the Calibration Curves of the Drug
Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Analysis of Capsule formulation
Assay of Capsule formulation

For analysis of the Capsule formulation (Emtriva 200mg), weight equivalent to weight 10 mg of Emtricitabine was transferred to 10 ml volumetric flask and dissolved in mobile phase. The solution was shaking vigorously for 20 mins and filtered through Whattman filter paper no.41, then volume was made up to mark with mobile phase. From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100µg/ml. From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 10 µg/ml of Emtricitabine. The amounts of Emtricitabine in Capsule formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with Capsule formulation.

Validation
Linearity
Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Accuracy
Recovery studies were performed to validate the accuracy of developed method. To pre analysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision
Repeatability
Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Intermediate Precision
Day to Day
Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods.

Robustness
As per ICH norms, small, but deliberate variations, by altering the pH and / or concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of emtricitabine was studied.

Detection limit and quantitation limit
The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Forced degradation studies
In order to determine whether the method is stability indicating, forced degradation studies were conducted on emtricitabine powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:
50 mg of emtricitabine sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of Emtricitabine.

Alkaline hydrolysis:
50 g of emtricitabine sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of emtricitabine.

Oxidative degradation:
50 mg of emtricitabine sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of emtricitabine.

Thermal degradation:
50 mg of emtricitabine sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of emtricitabine.

RESULT AND DISCUSSION:

Structure of Emtricitabine

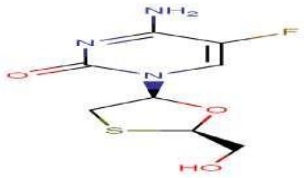

Figure 1: Structure of Emtricitabine

Result of FTIR of Emtricitabine

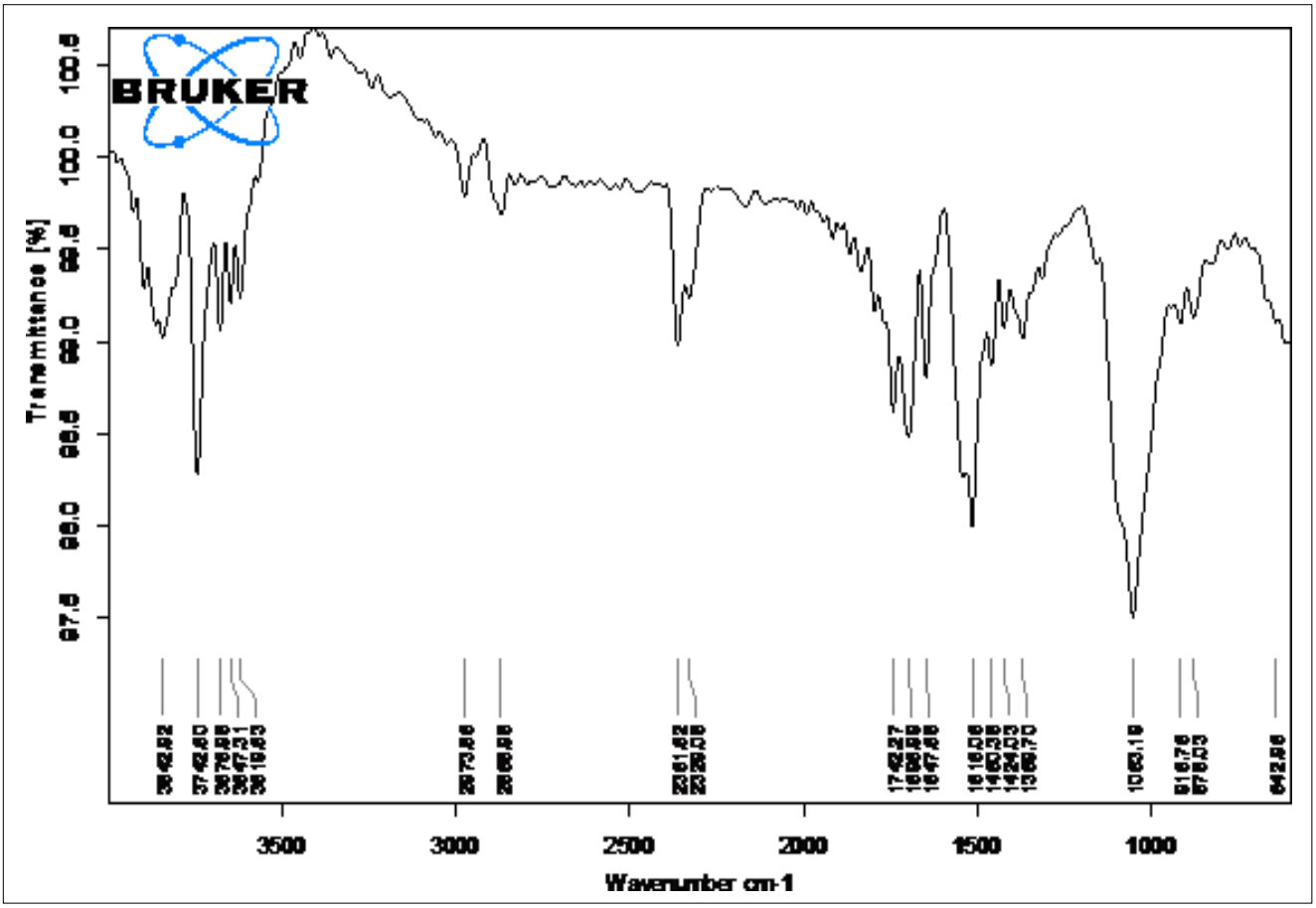

Figure 2: FTIR spectra of Emtricitabine

FT-IR Interpretation of Emtricitabine

Table 1: FT-IR Peak of Emtricitabine

S. No.	Peak Position	Interpretation
1.	2973.88	O-H stretching
2.	1742.27	C=O stretching
3.	1647.66	C=N stretching
4.	1063.18	C-F stretching

Method-RP-HPLC

Table 2 : Chemical Reagent used method development

Chemicals/Reagents	Grade	Company
Acetonitrile	HPLC	Merck
Methanol	HPLC	Merck
Potassium dihydrogen phosphate	AR	Rankem
Water	HPLC	Milli-Q
Triethanolamine	AR	Thomas Baker
Orthophosphoric acid	AR	Hi Media

Table 3: Instrument Specification

HPLC	Waters
Pump	515 Isocratic pump
Injector	Rheodyne injector with a 20-microlitre fixed loop
Detector	UV Vis detector
Software	Data ace software
Column	Thermo C-18 column (4.6 x 250mm, 5µ particle size)
Balance	Citizen (Cx-265)
Millipore	Mili- Q
Sonicator	PCI (Mumbai)
U.V. Vis. spectroscopy	Labindia 3000 Plus

Results of Solubility

Solubility of drug was observed by dissolving them in different solvents.

Table 4 : Solubility of drug in different solvents

S. No.	Solvent	Emtricitabine
1	Water	Soluble
2	Ethanol	Slightly Soluble
3	Acetonitrile	Soluble
5	0.1 N HCl	Freely soluble
6	0.1 N NaOH	Soluble
7	Chloroform	Insoluble
8	Methanol	Soluble

Results of HPLC method development

Determination of λmax

Accurately weighed 10 mg of drug was transferred into 10 ml volumetric flasks and dissolved in 10 ml of methanol and vortex it to get complete dissolution. From that 0.1 ml of stock solution dissolve in 10 ml of methanol which gives 10µg/ml of standard solution of Emtricitabine.

Selection of Mobile Phase

Initially to estimate Emtricitabine in fix dosage form number of mobile phase in different ratio were tried.

Table 5 : Mobile phase selection

Mobile Phase	Ratio	Retention Time
	Remark	
Methanol : Water	50 : 50 v/v	Tailing in peak
Acetonitrile : Methanol	50 : 50 v/v	Most suitable

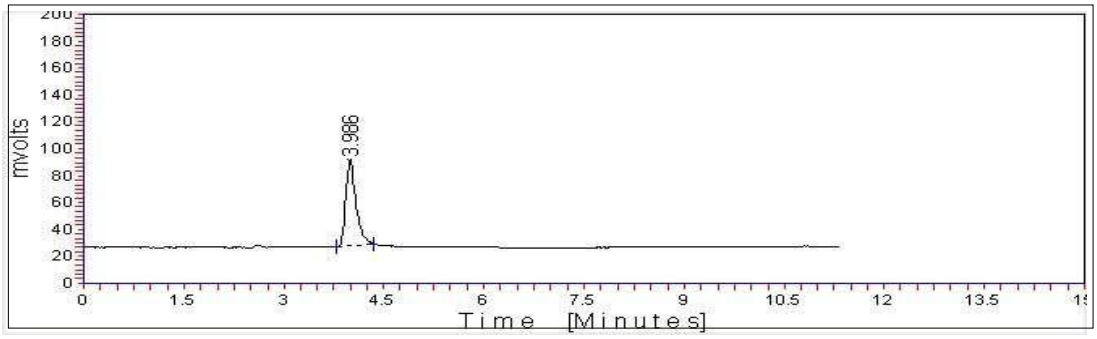

Figure 3: Mobile phase selection Acetonitrile: Methanol

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials mobile phase was used as diluents.

Selection of separation variable

Table 6 : Selection of separation variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5□
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Acetonitrile	50%
Methanol	50%
Diluent	Methanol
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 □l
Detection wavelength	254 mm
Retention time	
Emtricitabine	3.968 ± 0.3 min.

Preparation of the calibration curves of the drug

Each of the working standard solutions were injected 6 times and the mean peak area ratio of each drug to that of internal standard were calculated and plotted against the concentration of the drug. The regression of the concentration of each drug over the mean peak area ratio was obtained and these regression equations were used for the assay of Capsules containing these drugs.

Table 7: Linearity of Emtricitabine

Standard Concentration g/ml	Area under Curve (AUC)						Mean
	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	
0	0	0	0	0	0	0	0
5	125.658	132.458	124.789	129.658	120.365	128.785	126.95
10	255.698	268.951	279.985	245.658	240.785	250.147	256.87
15	367.852	360.745	372.658	365.854	379.985	352.159	366.54
20	489.985	496.658	475.658	480.741	475.621	479.985	483.11
25	612.258	618.854	610.478	619.985	610.478	615.325	614.56

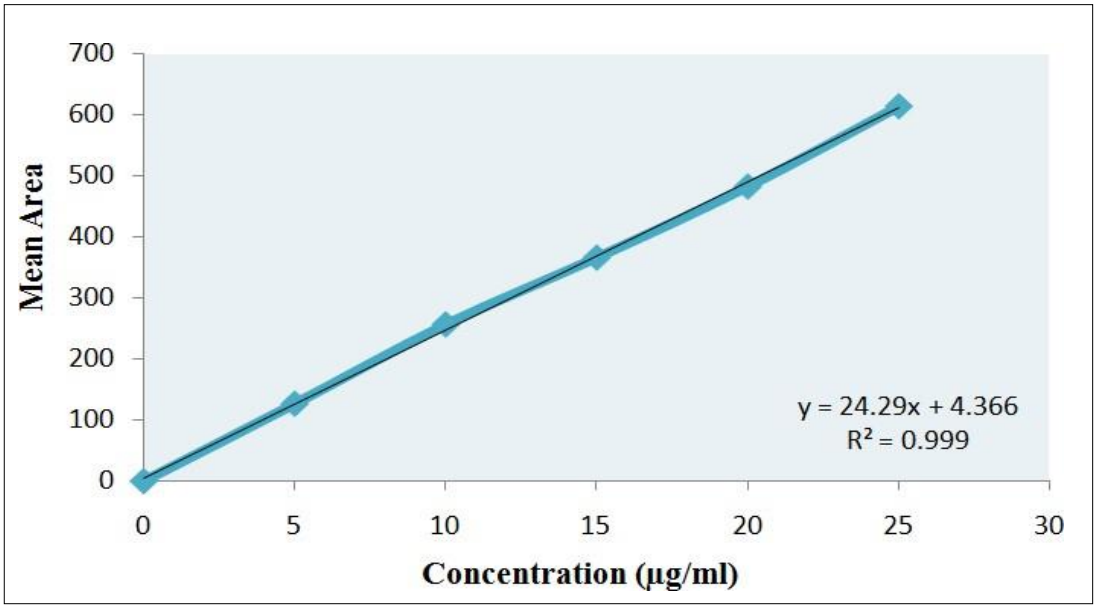

Figure 4 : Calibration Curve of Emtricitabine

Regression Equation

Y= mx+c,

AUC = 24.29 conc. + 4.366

Y= AUC
m= slope = 24.21
X= Conc. in □g/ml
c=Intercept4.36
r^2= 0.999

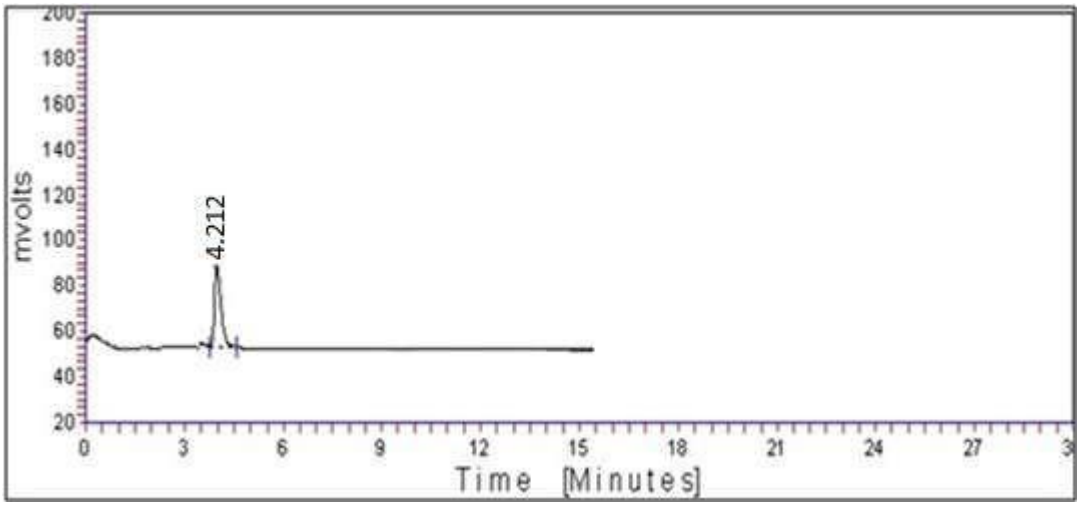

Figure 5 : Chromatogram of Emtricitabine

Analysis of Capsule sample

20 Capsules were taken and determine average weight, amount equivalent to 10mg of Emtricitabine was taken in 10 ml volumetric flask. The volume is made up to the mark by methanol and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of 10µg/ml concentration.

Table 8: Analysis of Capsule sample

S. No		Drug
		Emtricitabine
1.	Mean	99.45
2.	S.D.	0.215
3.	% RSD	0.220

Validation of developed method

Linearity

From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration. The Curve was plotted between response ratio Vs Concentration.

Table 9: Response Ration Data for Linearity of Emtricitabine

Replicates	Concentration (□g/ml)	Mean AUC	Response Ratio
Rep-1	5	126.95	25.39
Rep-2	10	256.87	25.69
Rep-3	15	366.54	24.44
Rep-4	20	483.11	24.16
Rep-5	25	614.56	24.58
Mean			24.85
S.D.			0.655
R.S.D.			2.637

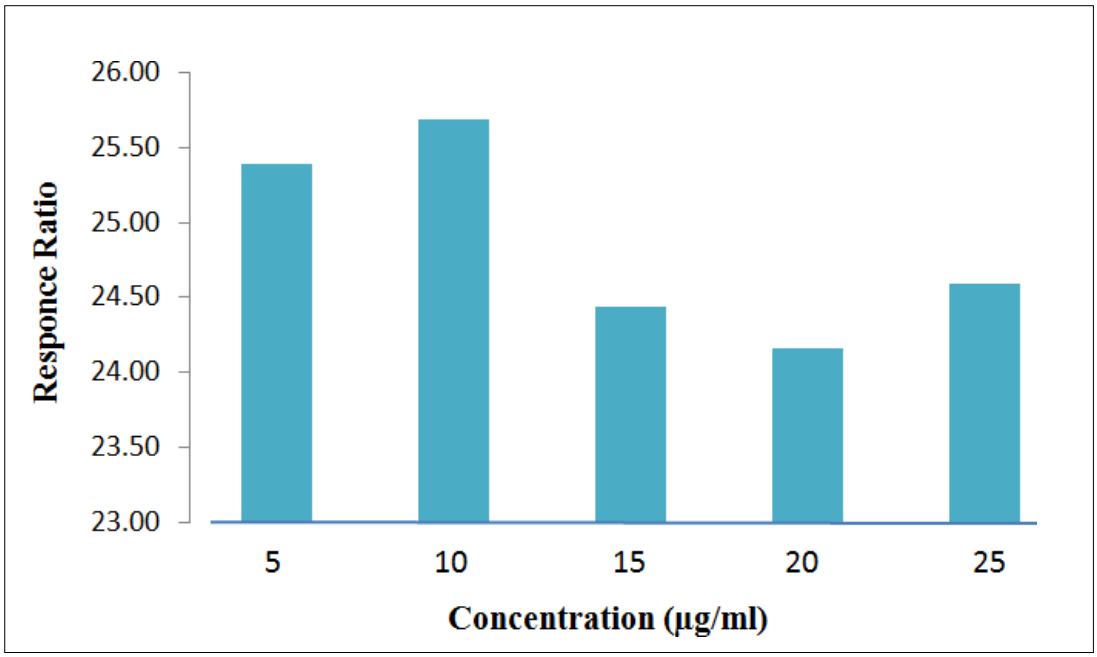

Figure 6 : Response Ration graph for linearity of Emtricitabine

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analysed sample solution, a definite concentration of standard drug was added and then its recovery was analyzed.

Table 10: Recovery study for accuracy of Emtricitabine

Conc. of drug in sample (mg)	**10**	**10**	**10**
Std drug added (mg)	**8**	**10**	**12**
Replicate 1	7.95	9.98	11.78
Replicate 2	7.92	9.65	11.89
Replicate 3	7.89	9.92	11.85
Mean	7.92	9.85	11.84
SD	0.030	0.176	0.056
%RSD	0.379	1.785	0.470
Mean % Recovery	99.00	98.50	98.67

Precision

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis.

Table 11: Result of repeatability for Emtricitabine

Conc. of drug in sample µg/ml	**5**	**10**	**15**	**20**	**25**
Replicate 1	4.89	9.95	14.85	20.02	24.85
Replicate 2	4.95	10.01	14.96	19.96	24.95
Replicate 3	4.96	9.98	14.95	19.85	24.78
Mean	4.933	9.980	14.920	19.943	24.860
SD	0.038	0.030	0.061	0.086	0.085
%RSD	0.767	0.301	0.408	0.432	0.344

Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Intermediate Precision: (A) Day to Day

Table 12: Result of intermediate precision for Emtricitabine

Conc. of Drug in sample µg/ml / **Replicate**	**5**	**10**	**15**	**20**	**25**
Replicate 1	4.98	9.85	14.85	19.95	24.85
Replicate 2	4.86	9.96	14.95	19.96	24.78
Replicate 3	4.93	9.84	14.96	19.85	24.65
Mean	4.923	9.883	14.920	19.920	24.760
SD	0.060	0.067	0.061	0.061	0.101
%RSD	1.224	0.674	0.408	0.305	0.410

Intermediate Precision: (B) Analyst to Analyst

Table 13 : Result of intermediate precision for Emtricitabine

Conc. of Drug in sample µg/ml Replicate	5	10	15	20	25
Analyst 1	4.98	9.98	14.85	19.98	24.78
Analyst 1	4.85	9.85	14.96	19.96	24.85
Mean	4.915	9.915	14.905	19.970	24.815
SD	0.092	0.092	0.078	0.014	0.049
%RSD	1.870	0.927	0.522	0.071	0.199

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Table 14: LOD and LOQ of Emtricitabine

Name	LOD (□g/ml)	LOQ (□g/ml)
Emtricitabine	0.35	1.05

Results of Forced Degradation studies

Table 15: Results of Forced degradation studies of Emtricitabine

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.15	0
Acidic hydrolysis	85.98	14.02
Alkaline hydrolysis	92.32	7.68
Oxidative degradation	89.65	10.35
Photolytic degradation	87.12	12.88

CONCLUSION:

The developed methods were found to be linear. The mean percent label claims of capsules by the proposed methods were close to 100, indicating the accuracy of the proposed method and low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method.

Table 1: Results of Linearity of Emtricitabine

S. No	PARAMETER	REMARK
1	Linearity	5-25µg/ml
2	Correlation Coefficient (r^2)*	0.999
3	Slope (m)*	29.29
4	Intercept (c)*	4.366

*Average of five determination

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curve was linear over the concentration range of 5- 25µg/ml and correlation coefficients were found to be 0.999 for Emtricitabine respectively.

Table 2: Results of Recovery Studies on Marketed Formulations

Recovery Level %	% Recovery (Mean±SD)*	% RSD
80	99.00±0.030	0.379
100	98.50±0.176	1.785
120	98.67±0.406	0.470

Recovery studies were carried out by applying the method to drug sample to which known amount of Emtricitabine at three concentration levels of 80, 100 and 120 % were added. At each level %recovery was determined, which are in the range of 98.67±0.406-99.00±0.030%. The results are given in Table 3.

Table 3: Results of validation (%R.S.D.)

PARAMETER		% RSD
Precision (%R.S.D.)*	Repeatability	0.767
	Day to Day	1.224
	Analyst to Analyst	1.870
	LOD	0.35
	LOQ	1.05

*Average of five determination

The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done by measuring the absorbance for six times. The % RSD value was found to be 0.767, 1.224, 1.870 for repeatability, day to day and analyst to analyst respectively indicating that the method is precise.

Modern medicines for human use are required to comply with specific standards and regulation set forth by the concerned authorities. The efficacy and safety of medicinal products can only be assured by analytical monitoring of its quality. Pharmaceutical analysis is an art and science of determining the concentration of drug constituents present in marketed formulation. It is considered as an application of procedures necessary to determine and estimate the identity, strength, quality and purity of drug. Therefore, the quality control laboratory is considered as the backbone of the Pharma industries with ever-increasing need for the development of analytical techniques for drug formulation.

In the present study, a successful attempt was made for the HPLC quantitative estimation of emtricitabine in bulk formulation.

The method was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. The entire work was performed on waters HPLC with U.V.Vis detector

The result obtained shows the developed method to be precise, simple, rapid and accurate. Thus these can be used for routine analysis of Emtricitabine in bulk drug and dosage form.

It was thus, concluded that the proposed method is new, simple, accurate, safe, free form pollution, precise and can be successfully employed in the routine analysis. The simplicity, rapidity reproducibility and economy of the proposed methods completely fulfill the objective of this research work.

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