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UPLC method for the quantitative estimation of emtricitabine impurities in formulated drug product

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ABSTRACT

A simple, faster reverse phase UPLC method has been developed for quantitative estimation of Emtricitabine impurities in pharmaceutical dosage form. Emtricitabine is an antiretroviral compound that inhibits the HIV-1 reverse transcriptase. Chromatographic separation was achieved on Waters Acquity UPLC BEH C8 , 100x 2.1 mm ,1.7µm column in a gradient mode elution. Detection wavelength was set at 270 nm. Drug product was subjected for stress conditions of Acid, Alkali and Peroxide degradation. Emtricitabine was found to have degraded significantly in peroxide condition. Peak purity results of Emtricitabine indicated that all degradents are separated from the analyte peak. The developed method was validated as per ICH guidelines with respect to Specificity, Linearity, Accuracy, Precision, Limit of detection and quantification.

Key words: Emtricitabine, Impurities, UPLC, Forced degradation.

INTRODUCTION

Emtricitabine is an antiretroviral compound that inhibits the HIV-1 reverse transcriptase. Emtricitabine is chemically 5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine [1] . Literature survey revealed that several methods have been reported for determination of assay of Emtricitabine. Few methods have been reported for the estimation of impurities of Emtricitabine by HPLC [1-11]. However, there are no methods reported to determine of Emtricitabine by Ultra Performance Liquid Chromatography(UPLC). Hence an attempt was made to develop and validate a UPLC method for the estimation of impurities of Emtricitabine in formulated drug product. Ultra Performance Liquid chromatography is a relatively new technique in the field of Liquid chromatography. UPLC helps in improving speed , sensitivity , resolution and reduction in solvent consumption. UPLC makes use of stationary phase with particle size less than 2µm.Instrumentation has been designed to accommodate high pressure and high temperatures as low particle size phases are used [13-14].

The impurities incorporated in the study are Carboxylic acid, S-oxide, Lamivudine and Des amino [Fig 1]. Forced degradation studies in Acid, Alkali and Peroxide conditions by using PDA detector were performed to ensure that degradents were separated from analyte peak.

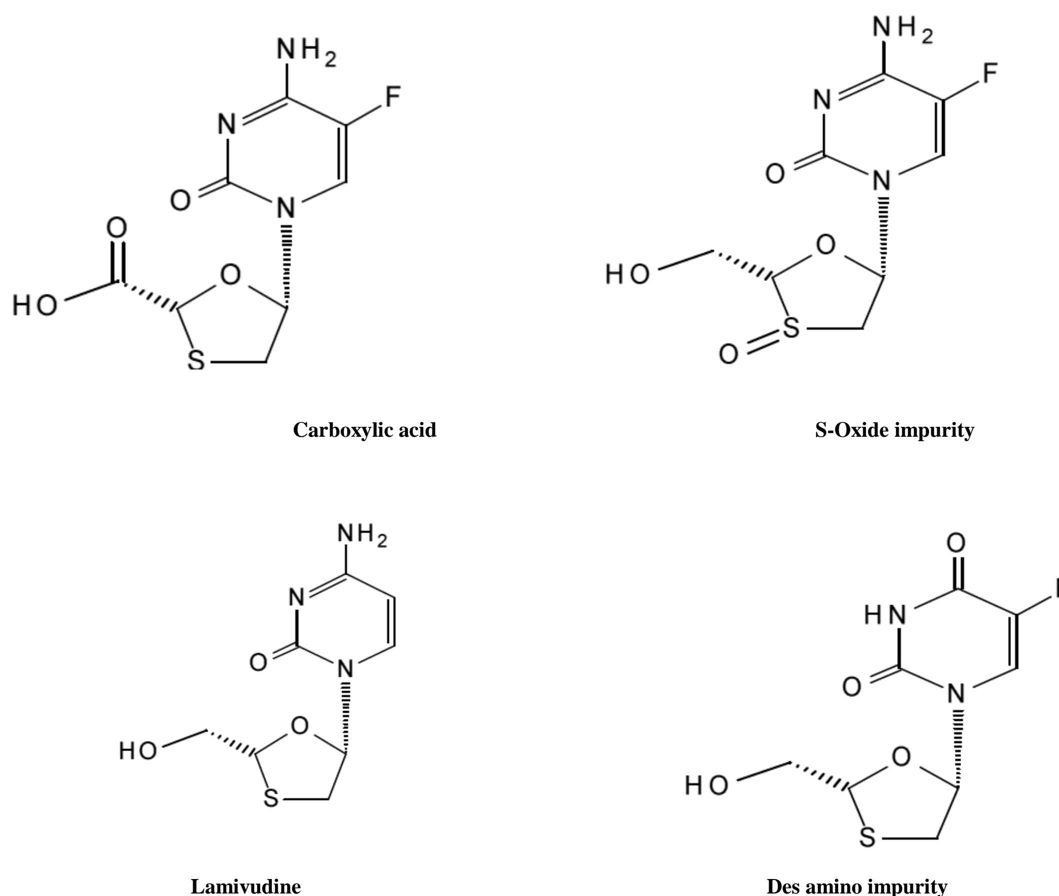

Fig 1. Chemical structure of Emtricitabine impurities

MATERIALS AND METHODS

2.1 Materials : Chemicals and reagents:

Ammonium acetate used was of Analytical reagent grade from Merck chemicals. HPLC grade Methanol was from Rankem chemicals and HPLC grade water from Millipore Milli Q Water purification system were used throughout the experiment.

2.2 Equipment:

Ultra performance Liquid chromatography system (from Waters) with auto sampler and with Photo Diode Array detector was used for the study. Data was acquired and processed by using Waters Empower software.

2.3 Chromatographic Conditions:

The analysis was carried out on Waters Acquity UPLC BEH C8 , 100x 2.1 mm ,1.7µm column. The column oven temperature was maintained at 40°C. The mobile phase A consists of 0.01 M ammonium acetate buffer with pH 4.8 . A mixture of Methanol and buffer in the ratio of 80:20 was used as Mobile phase B. The flow rate was set to 0.4 mL/minute in gradient elution mode. Gradient time program was set as T/%B: 0/0, 5/0, 8.5/10, 10/53, 11.5/85, 13.5/15, 14/0, and 17/0. The injection volume was 2µL and the detection was performed at 270nm using a photo diode array (PDA) detector. The typical retention time of Emtricitabine is about 6.9 minutes in the final optimized conditions. The criticality of this method is to elute impurities of Emtricitabine with optimum separation and symmetric peak shapes with no interference due to placebo.

2.4 Sample Preparation:

2.4.1 Diluent Preparation:

A Mixture of Water and Methanol in the ratio of 80:20 %v/v was used as diluent.

2.4.2 Standard Preparation :

A standard solution was prepared to get a concentration of 3µg/mL Emtricitabine. Accurately weigh and transfer 30mg of Emtricitabine standard into a 100 mL volumetric flask added 70 mL of diluent , dissolved and diluted to

volume with diluent. Transferred 1.0 mL of above solution into a 100mL volumetric flask, diluted to volume with diluent .

2.4.3 Test Preparation:
Test solution was prepared by taking homogenous mixture of formulated powder equivalent to 60 mg of Emtricitabine into a 100mL volumetric flask. Added about 30 mL of diluent, sonicated for about 20 minutes , and made up to the volume with diluent and filtered through a 0.45µm membrane filter.

2.5 EXPERIMENTAL DESIGN:
2.5.1 Method Validation:
The principal purpose of analytical method validation is to ensure that selected analytical procedure will give reproducible and reliable results that are adequate for the intended purpose as described in ICH guidelines [12]. The described method has been validated in terms of specificity, precision, linearity, accuracy, Limit of Quantification and detection. Specificity of the method was evaluated by injecting individual impurities, placebo and by subjecting the drug into forced stress conditions. Linearity of the method was statistically proved by correlation. The precision of the method was expressed in term of coefficient of variation (RSD) for % of impurities. The accuracy was expressed in terms of percent recovery of the known amount of impurities added to the sample preparation.

RESULTS AND DISCUSSION

3.1 System suitability:
Checking suitability of the system before any analysis is an integral part of a liquid chromatographic methods. As integral part of chromatographic method, system suitability parameters like USP Tailing, Theoretical plates and Relative standard deviation (RSD) for replicate injections of standard were evaluated and found to be satisfactory as per common chromatographic practices. Results are shown in Table No 1.

Table 1: Results of System Suitability Test

Name of Drug substances	%RSD for replicate injections	USP Tailing factor	Theoretical plates
Emtricitabine	0.7	1.1	18189

3.2 Specificity:
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradents, matrix (placebo) etc. Specificity was tested by injecting the impurity standards individually, Test spiked with all impurities (Fig 2), placebo preparation and Forced degradation samples. Results of impurity interference study has been tabulated in Table No 2.

Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Forced degradation was attempted to stress conditions like acid hydrolysis, base hydrolysis, and oxidative degradation. To check and ensure the homogeneity (peak purity) of peak in the stressed sample solutions, photo diode array detector was employed. In forced degradation study it was observed that Emtricitabine is susceptible to degradation in peroxide and base stress conditions. Peak purity in all the degradation conditions has been proven for Emtricitabine peak. Results are tabulated in Table No 3.

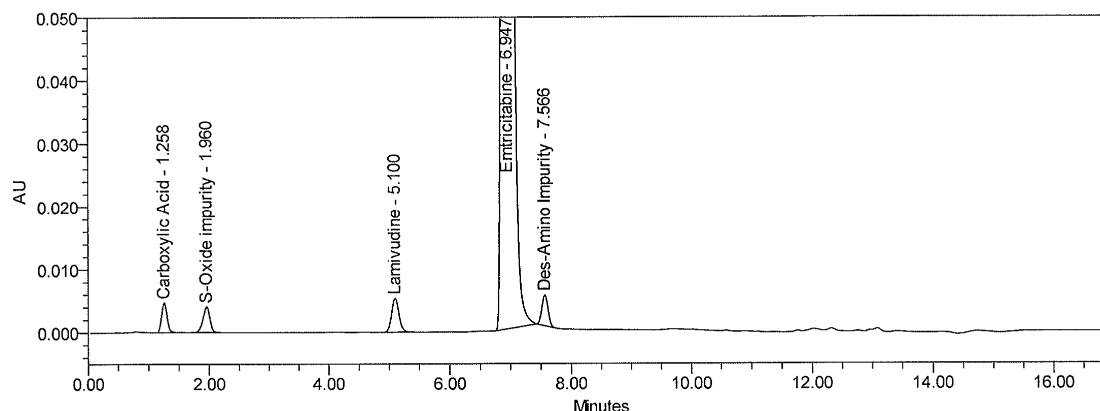

Fig. 2 Typical Chromatogram of Test Spiked with impurities

Table 2: Results of Specificity - Impurity Interference

Impurity Name	RT (minutes)
Carboxylic acid	1.25
S-Oxide Impurity	1.96
Lamivudine	5.10
Des amino impurity	7.56
Emtricitabine	6.94

Table 3: Results of Forced degradation Studies with Peak purity details

Stress Conditions	PA	PT	% Degradation
Acid Degradation	0.102	0.253	0.3
Base Degradation	0.102	0.252	0.9
Peroxide Degradation	0.109	0.252	6.1

PA = Purity Angle, PT= Purity Threshold

***Note**: Purity Angle should be less than Purity Threshold to meet Peak purity criteria acceptance criteria*

3.3 Linearity:

The linearity of the analytical procedure was demonstrated to prove the proportional relationship of response versus concentration over the range. It is common to perform linearity experiments over a wide range of impurity concentration covering from about LOQ to higher level. This gives confidence that the response and concentration are proportional and consequently ensures that calculations can be performed in the specified range. The linearity of detector response to different concentrations of all impurities of Emtricitabine was studied by preparing a series of solutions. The data were subjected to statistical analysis using a linear-regression model. The results have indicated good linearity. Results are shown in Table No 4.

Table 4: Results of Linearity Studies (Response Vs Concentration)

Name of Impurities	Correlation coefficient	Intercept	Slope
Carboxylic acid	0.999	158.527	6192.689
S-Oxide Impurity	0.999	308.798	8709.803
Lamivudine	0.999	61.543	12433.3
Des amino impurity	0.999	362.914	8911.761

3.4 Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions . Six sample solutions were prepared spiking the known impurities and the precision of the method was tested. The % RSD was calculated for results of all impurities and it indicates that proposed method has got acceptable level of repeatability. Results are tabulated in Table No 5.

Table 5. Method Precision data

Impurity Names	Mean from Six samples (% Impurity)	% RSD for six samples
Carboxylic acid	0.601	0.5
S-Oxide Impurity	0.540	0.8
Lamivudine	0.525	0.7
Des amino impurity	0.540	0.4

Table 6: Results of Recovery Study at Different Levels

Name of Impurity	50% level	100% level	150% level
Carboxylic acid	103.0	103.5	102.4
S-Oxide Impurity	105.4	90.5	93.7
Lamivudine	107.1	104.9	106.8
Des amino impurity	97.7	102.9	104.1

Note: Number of samples analyzed at each level is in triplicate

3.5 Accuracy:

Accuracy of an analytical method is the closeness of the test results obtained by the method to that of true value. Accuracy of the proposed method was established by recovery experiments. This study was employed by spiking of known amounts of impurities into samples of at 50%, 100% and 150% of targeted concentration, in triplicate and

injected into the chromatographic system. The resulting mixtures were analyzed as described in proposed method. Results obtained from recovery studies are given in Table No 6.

3.6 Limit of Quantification (LOQ):

The limit of quantification (LOQ) and Limit of detection (LOD) for impurities was determined by signal to noise ratio method. concentration of impurities which gave Signal to ratio about 10 is considered as LOQ and concentration of impurities which gave Signal to ratio about 3 is considered as LOD. Results obtained from this study is given in Table No 7.

Table 7: Results of LOD and LOQ

Name of Impurity	S/N ratio		% Impurity	
	LOQ	LOD	LOQ	LOD
Carboxylic acid	11.3	3.5	0.034	0.011
S-Oxide Impurity	9.6	3.1	0.037	0.009
Lamivudine	13	3.2	0.030	0.007
Des amino impurity	11.3	3.3	0.028	0.008

From all the above validation parameters performed ,it indicates that method is specific and selective, precision of the all the impurities was found be less than 1% RSD, correlation was found to be 0.999 for all the impurities, recovery results were within the acceptable limits and LOQ value is less than the reporting threshold. This proves that method is specific, precise ,linear and accurate.

CONCLUSION

A simple, faster and economical UPLC method could be developed for the quantitative estimation of impurities of Emtricitabine in formulated product. Method requires all commonly available materials for analysis. Analytical method was validated as per ICH guideline and proved that it is suitable for its intended purpose.

The above validated UPLC method can be used by government agencies, government laboratories, research institutions and manufacturing companies to analyze the drug product to check the quality of it.

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