RESEARCH ARTICLE

Estimation of Glyoxylic Acid in Emtricitabine by a New Validated RP-HPLC Method

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ABSTRACT

To establish a simple, sensitive, as well as specified method for the separation and quantitative determination of glyoxylic acid in emtricitabine by using reversed-phase high-performance liquid chromatography (RP-HPLC). A superior separation of glyoxylic acid in emtricitabine was done by using a C8 column and a mobile system of (0.1% OPA and ACN: H_2O) with 1-mL/min flow rate in gradient mode and a wavelength of 210 nm. The glyoxylic acid was eluted at 5.37 minutes with good efficiency and system suitability. The method has shown good linearity ranges from a limit of detection (LoQ) to 150% level of glyoxylic acid standard solution (0.1% or 0.001 mg/mL). ICH principles validated the suggested approach, and every parameter met the requirements for the International Council of Harmonization (ICH) Q2 recognition. %RSD of System, method precision, and precision at LoQ were in the range of 0.46 to 1.91. The developed method was sensitive, specific, and precise in estimating the trace levels of glyoxylic acid in emtricitabine.

Keywords: Glyoxylic acid, Emtricitabine, Sensitive, Gradient elution, Specificity.

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INTRODUCTION

Since the beginning of the 21st century, researchers and professionals in the medical field have been trying to find an effective therapy for human immunodeficiency virus (HIV) infection. Up to this point, the Food and Drug Administration (FDA) has given its stamp of approval to over 20 different antiviral medications that can treat this infection.[1] Emtricitabine's antiviral efficacy has been attributed to its metabolite emtricitabine 5'-triphosphate, which efficiently inhibits the reverse transcriptase enzyme and prevents HIV replication.[2] Glyoxiclic acid is a ketone that contains carboxylic acid and has a significant role in plants and human beings.[3] Figure 1 displays the chemical structure of glyoxylic acid as well as emtricitabine

A comprehensive literature analysis of emtricitabine revealed the existence of a variety of analytical techniques, including UV methods and reversed-phase high-performance liquid chromatography (RP-HPLC) techniques, which may be used to determine the concentration of emtricitabine both on its own and in combination with other pharmacological substances.[4-6] In addition to that, a small number of RP-HPLC, as well as liquid chromatography-mass spectrometry (LC-MS) methods, have been published regarding the detection of emtricitabine and the process and product impurities that are associated with it in bulk and dosage form.[7-11] There has not been a solitary method that has been described that can separate, identify, and quantify the glyoxylic acid that is present in emtricitabine using RP-HPLC. As a result, we have been doing research to create an effective, sensitive, and economical RP-HPLC method to determine the concentration of glyoxylic acid in the medication emtricitabine. The approach that was created was validated by the provisions of ICH Q2 (R1).

MATERIALS AND METHODS

Fortune Pharma, located in Hyderabad, donated gift samples of active pharmaceutical components for emtricitabine. Both

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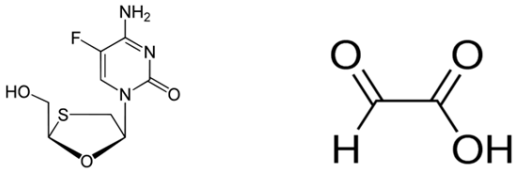

Figure 1: Chemical structures of emtricitabine and glyoxylic acid

the water and the acetonitrile of HPLC quality were purchased from Finar Chemicals. Fortune Pharma, located in Hyderabad, donated gift samples of active pharmaceutical components for emtricitabine. Both the HPLC-grade acetonitrile and the water used in the experiment were purchased from Finar Chemicals.

Chromatographic Conditions

The RP-HPLC procedure was performed using a WATERS 2695 equipped with a PDA detector and an auto-sampling system. Effective separation was achieved by injecting 20 L of the standard solution of glyoxylic acid into a Symmetry C18 (250 x 4.6 mm, 5 m) column. The mobile phase was composed of solvent A (0.10% orthophosphoric acid in water) and solvent B (acetonitrile: water in a 90:10 v/v ratio), and it was applied in a gradient manner. The flow rate was set at 1-mL/min. At a wavelength of 210 nm, the eluted analytes gave an effective response both subjectively and quantitatively. The gradient schedule for the mobile phase is presented in Table 1.

Preparation of Standard Solution

In a 50 mL volumetric flask, 10 mg of pure glyoxylic acid has been weighed, transferred, and then dissolved in a diluent. Shaking the flask and adding diluents to the appropriate volume produced a stock solution-1 with a 0.2 mg/mL concentration. To produce a 0.001 mg/mL solution, 10 mL of the standard stock solution was diluted with the diluent.

Preparation of Sample Solution

A 50 mg of emtricitabine was weighed and dissolved in 10 mL of volumetric flask with diluents to obtain 5.0 mg/mL. The 0.25 µm nylon filter was used to filter the sample solution.

Method Validation

The method has been developed and validated per ICH Q2 requirements.[13-15]

System suitability test

The present RP-HPLC technique was tested for system appropriateness by injecting a reference solution containing glyoxylic acid inside the HPLC analyzer six times in succession. Recorded chromatograms had been interpreted for the assessment of the %RSD of the peak areas were done for the recorded chromatograms.

Linearity

Linearity was confirmed by computing the R^2 value of an array of concentrations from LoQ to 150% level of glyoxylic acid standard solution (0.1% or 0.001 mg/mL) by constructing a linear plot linking the series of concentrations mentioned for each analyte and their corresponding peak areas. Each concentration level was injected three times and the average peak area was used to construct a linear graph.

Table 1: Gradient programme of method

Time (Min)	*Solvent-A (%v/v)*	*Solvent-B (%v/v)*
0.01	97	3
10.0	97	3
15.0	5	95
25.0	5	95
27.0	97	3
35.0	97	3

Precision

Validation of system precision of the stated approach was performed by injecting 0.1% glyoxylic acid in 6 repeated injections. The %RSD was calculated for each of the duplicated injections acquired peak regions. Method precision of the optimized method was done by injecting a solution consisting of emtricitabine solution spiked with 0.1% glyoxylic acid solution. The %RSD of the recovery for each was calculated.

Accuracy

To make sure about the accuracy of the current HPLC method, a %recovery procedure was chosen, in which emtricitabine sample solutions were spiked with glyoxylic acid in three separate levels: 50, 100, and 150%. Three serial injections of each spiked solution were introduced into HPLC and assessed the %mean recovery of glyoxylic acid in the spiked solution.

Specificity

The specificity of the current approach was confirmed by injecting successive individual injections of blank glyoxylic acid solution and a solution of glyoxylic acid spiked with emtricitabine. The chromatograms produced were interpreted to observe any interference at the RT of glyoxylic acid impurity.

Sensitivity

The S/N ratio determined the LoD and LoQ. In general, the quantification amount is three times more than the detection limit.

Robustness

To confirm the robustness of the HPLC technique, slight variations were produced during method conditions with intention. Slight modifications were made to attributes such as the flow rates (± 0.1 mL/min), and wavelength (λ_{max}) (± 2 nm). The %RSD of the obtained peaks was determined.

RESULT AND DISCUSSION

Method Optimization

To obtain a chromatogram with satisfactory system suitability parameter values, various mobile system compositions that had different ratios, various kinds of columns, and altering of the flow rate were tried. This trial-and-error process was used to determine the optimized method. Finally, the method using Symmetry C18, (250x4.6 mm, 5 µm) column with a mobile phase composition of solvent A (0.1% orthophosphoric acid in

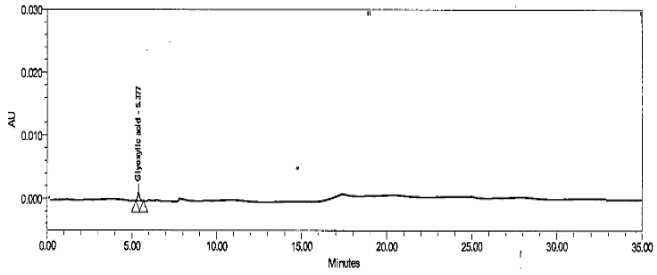

Figure 2: Optimized method chromatogram of glyoxylic acid

Table 2: System suitability test of glyoxylic acid

Injection replicates	*Peak area of glyoxylic acid*
1	11286
2	11240
3	11192
4	11208
5	11140
6	11173
Mean	11207
%RSD	0.46

water) and solvent B (acetonitrile: water in 90:10 v/v ratio) in gradient manner and flow rate of 1-mL/min was considered as an optimized method. The glyoxylic acid was eluted at 5.7 minutes with good efficiency Figure 2.

Method Validation

System suitability

The data reported indicate that the method meets the system appropriateness requirements. Thus, the method validation condition is satisfied by the system appropriateness parameter. The %RSD value of six replicate injections did not deviate from the acceptability limits (≤2) of the ICH Q2 standards, as indicated in Table 2.

Linearity

The regression coefficient (R^2) value obtained from the calibration curve was 0.999 for the concentration ranges from 50 to 150% LoQ level of glyoxylic acid standard solution (0.1% or 0.001 mg/mL) claims that the approach exhibits a high degree of linearity for the given concentration series that was presented. The outcomes of the linearity tests shown in Table 3 and Figure 3:

Accuracy

At least three bioanalytical batches were examined to identify differences both within and between batches. The corresponding calibration curve will be used to calculate the QC sample concentration. The %recovery of glyoxylic acid in spiked solutions of glyoxylic acid was assessed to be 100 ± 10% (Table 4), which extensively unveils the accuracy of the HPLC method as per ICH limits.

Precision

The assessed %RSD values for the peak areas of the glyoxylic acid in 0.1% glyoxylic acid solution were ≤ 2 on multiple replications. 0.46, 0. and 1.91 are the system, method, and LoQ precision's %RSD. As a result, the HPLC technique yields accurate results. Table 5 provides analytical data for glyoxylic acid with their precisions.

Table 3: Linearity results of glyoxylic acid

Level	*Concentration (%)*	*Average peak area of glyoxylic acid*
LoQ	0.020	2018
50	0.051	5413
75	0.076	8003
100	0.102	10561
125	0.127	13384
150	0.153	16212
Correlation coefficient		0.9998

Sensitivity

The signal-to-noise (S/N) ratio for the LoD and LoQ were computed. As per ICH guidelines Q2 (R1), the slopes of three developed calibration curves and the standard deviations of the responses were used to determine the LoD and LoQ. The calculated values of LoD and LoQ of glyoxylic acid were shown in Table 6.

Specificity

No interference with the RT of glyoxylic acid was seen by blank and emtricitabine in the recorded chromatograms, which

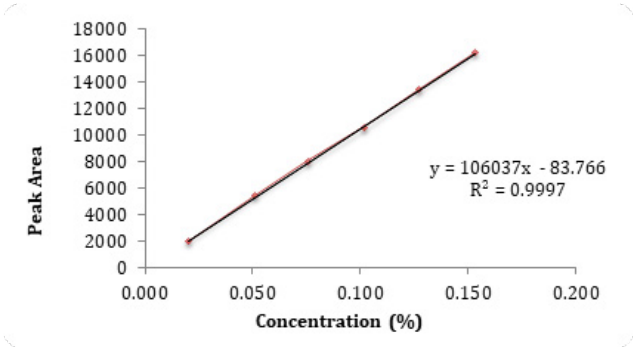

Figure 3: Linearity plot of glyoxylic acid

Table 4: Results of percentage recovery

Accuracy level	*Preparations*	*%Recovery of glyoxylic acid*
LoQ	1	93.6
	2	93.6
	3	93.6
50%	1	90.8
	2	90.2
	3	91.6
100%	1	89.1
	2	90.0
	3	89.7
150%	1	91.7
	2	91.6
	3	91.5

Table 5: Results of precision of glyoxylic acid

Injection replicates	*System precision*	*Method precision*	*LoQ precision*
1	11286	0.091	2007
2	11240	0.092	2029
3	11192	0.091	2059
4	11208	0.092	1994
5	11140	0.092	2085
6	11173	0.091	1987
Mean	11207	0.092	2027
% RSD	0.46	0.54	1.91

Table 6: Results of LoD and LoQ

Parameter	*Concentration with respective Test (%)*	*S/N ratio*
LoD	0.006	4.0
LoQ	0.02	13.7

Table 7: Robustness results of 0.1% glyoxylic acid solution

Parameter		*Peak area (Mean ± SD), N = 6*	*%RSD*
Flow rate (mL/min)	1.0	11216 ± 158.14	1.43
	0.9	11250 ± 149.62	1.32
	1.1	11172 ± 163.11	1.47
Wavelength (nm)	210	11201 ± 161.29	1.43
	208	11138 ± 159.27	1.43
	212	11162 ± 158.50	1.41

unveils the specificity of the stated HPLC method towards the analysis of glyoxylic acid only.

Robustness

The method's robustness was demonstrated by the fact that it was unaffected by slight changes in wavelength and flow rate (Table 7).

Trace levels in process impurities or foreign substances in the pharmaceutical can cause health consequences to concern patients. It is mandatory to identify such impurities before marketing the pharmaceuticals. Glyoxylic acid is one of the major process impurities of emtricitabine synthesis. The stated method can't adroitly separate and identify the glyoxylic acid in emtricitabine. Every parameter's response standard deviation was determined, and when the percentage RSD was less than 2%, it was concluded that the procedure was reliable.

CONCLUSION

The glyoxylic acid in emtricitabine in bulk and tablet form was determined using a simple and accurate RP-HPLC technique. Based on the information gathered, it was determined that no technique existed previous to the suggested approach. The devised technique has a suitable Glyoxylic acid elution time (5.37min) and good sensitivity. To determine the glyoxylic acid in emtricitabine, the technique comprises specificity and precision. As a result, the suggested approach is well-suited for use in the pharmaceutical industry.

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