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R E S E A R C H A R T I C L E

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Emtricitabine and Lamivudine in Pharmaceutical Dosage Form

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A B S T R A C T

A new method was established for simultaneous estimation of a Emtricitabine and Lamivudine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Emtricitabine and Lamivudine by using ZODIAC –SIL RP C18 4.6×100 mm 3.0µm column, flow rate was 1.0 ml/min, mobile phase ratio was (75:25 v/v) acetonitrile : phosphate buffer (KH_2PO_4 and K_2HPO_4) pH 2.5 (pH was adjusted with orthophosphoric acid), detection wave length was 292 nm. The instrument used was Shimadzu, UV detector, LC solutions. The retention times were found to be 2.746 mins and 3.668 mins. The % purity of Emtricitabine and Lamivudine was found to be 99.95% and 100.63% respectively. The system suitability parameters for Emtricitabine and Lamivudine such as theoretical plates and tailing factor were found to be 3923, 1.43and 3348 and 1.46, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Emtricitabine and Lamivudine was found in concentration range of 5µg/mL-25µg/mL and 5µg/mL-25µg/mL and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 1.67 and 1.48, % RSD for intermediate precision was 1.83 and 1.05 respectively. The precision study was precise, robust, and repeatable. LOD value was 0.110 and 3.0, and LOQ value was 0.33 and 9.09 respectively.

Key words: Emtricitabine, Lamivudine, RP-HPLC, Acetonitrile.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Emtricitabine (commonly called FTC, systematic name 2',3'-dideoxy-5-fluoro-3'-thiacytidine), with trade name Emtriva (formerly Coviracil), is a nucleoside reverse-transcriptase inhibitor (NRTI) for the prevention and treatment of HIV infection in adults and children.

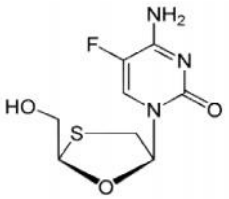

Fig 1: Structure of Emtricitabine

Lamivudine, commonly called 3TC, is an antiretroviral medication used to prevent and treat HIV/AIDS. It is also used to treat chronic hepatitis B when other options are not possible. It is effective against both HIV-1 and HIV-2. It is typically used in combination with other antiretrovirals such as zidovudine and abacavir. Lamivudine may be included as part of post-exposure prevention in those who have been potentially exposed to HIV.

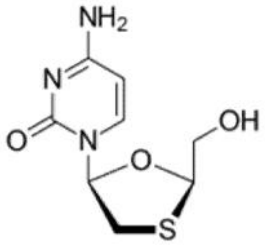

Fig 2: Structure of Lamivudine

2. Materials and Methods

Instrumentation:

SystemShimadzu, Pump Analytical HPLC isocratic pump, Detector UV detector, Software LC solutions software, ZODIAC –SIL RP C18 4.6×100 mm 3.0µm column, Sonicator SE60US, U.V double beam spectrophotometer T60, UV win 5 pH meter AD 102U, Weighing machine ER 200A Ascoset.

Chemicals:

Emtricitabine and Lamivudine, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Optimized chromatographic conditions

Column : Zodiac sil RP C18 4.6×100mm 3.0µm
Mobile phase ratio : ACN: pH 2.5 buffer (75: 25 % v/v)
Detection wavelength: 292nm
Flow rate : 1.0 ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature: Ambient
Run time : 8 min
Retention time : 2.764 and 3.668 mins.

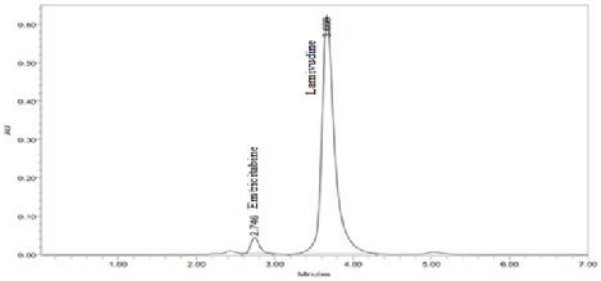

Fig 3: Chromatogram from optimized conditions

Observation: The retention time of both peaks was good response and height of peaks was good.

Sample solution preparation:

20.02 mg of Emtricitabine and 30.02mg of Lamivudine tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 1.5 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluents.

Standard solution preparation:

10 mg of Emtricitabine and 10 mg of Lamivudine working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1.5 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

- ✓ System Suitability
- ✓ Linearity
- ✓ Specificity
- ✓ Precision (Repeatability & Intermediate precision)
- ✓ Accuracy
- ✓ Limit of Detection and Limit of Quantification
- ✓ Robustness

3. Results and Discussion

Table 1: Results for system suitability

S.No	Peak Name	R_t	Area	Height	USP plate count
1	Emtricitabine	2.746	10966728	1412054	3445
2	Lamivudine	3.668	1397231	177886	5441

Table 2: Linearity results for Emtricitabine

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	221543

2	II	10 ppm	426277
3	III	15 ppm	624999
4	IV	20 ppm	826124
5	V	25 ppm	1022139
Correlation Coefficient			0.999

Table 3: Linearity results for Lamivudine

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	211543
2	II	10 ppm	426277
3	III	15 ppm	634999
4	IV	20 ppm	846124
5	V	25 ppm	1042139
Correlation Coefficient			0.999

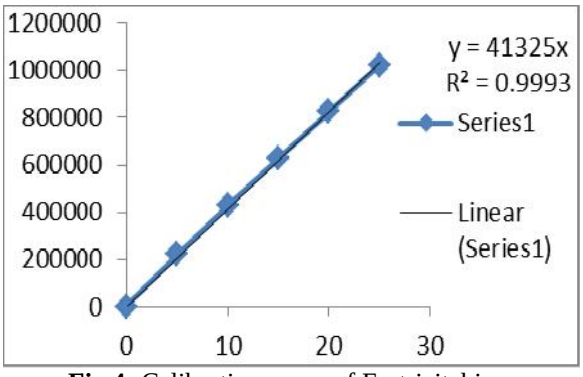

Fig 4: Calibration curve of Emtricitabine

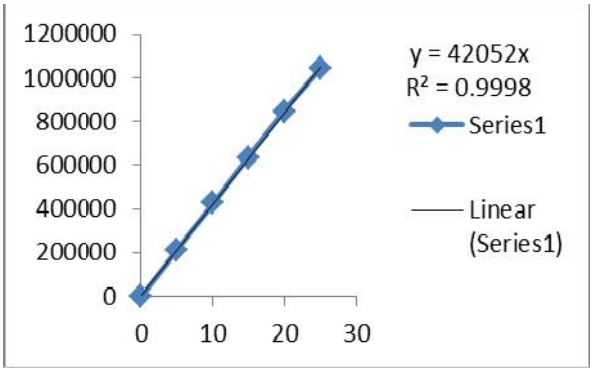

Fig 5: Calibration curve of Lamivudine

Table 4: Showing accuracy results for Emtricitabine

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	7371253	5	4.9	99.91%	99.56%
100%	14634226.7	10	9.98	99.18%	
150%	2243270.7	15	14.89	99.60%	

Table 5: Showing accuracy results for Lamivudine

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	5.0	4.9	99.53%	99.48%
100%	967998	10.0	9.59	99.38%	
150%	145437	15.0	14.85	99.52%	

Table 6: Showing% RSD results for Emtricitabine

	Name	RT	Area	Height(v)
1	Emtricitabine	2.729	115191	17639
2	Emtricitabine	2..723	110395	16008
3	Emtricitabine	2.728	113883	16394
4	Emtricitabine	2.728	111611	16507
5	Emtricitabine	2.726	112693	16386
Mean			112693	
Std.Dev.			1884.2	
%RSD			1.67	

Table 7: Showing %RSD results for Lamivudine

	Name	RT	Area	Height(v)
1	Lamivudine	3.665	2929297	280493
2	Lamivudine	3.667	2871804	245324
3	Lamivudine	3.670	2981706	253065
4	Lamivudine	3.668	2883219	248703
5	Lamivudine	3.665	2920005	258365
Mean			2917206	
Std.Dev.			43389.9	
%RSD			1.48	

Table 9: Showing intermediate precision results for Emtricitabine

	Name	RT	Area	Height(v)
1	Emtricitabine	2.729	105191	17601
2	Emtricitabine	2.723	100395	16000
3	Emtricitabine	2.728	103883	16286
4	Emtricitabine	2.728	101611	16486
5	Emtricitabine	2.726	102386	16275
Mean			102693	
Std.Dev.			1884.2	
%RSD			1.83	

Table 10: Showing intermediate precision injection

	Name	RT	Area	Height(v)
1	Lamivudine	3.665	2829297	280486
2	Lamivudine	3.667	2871804	245316
3	Lamivudine	3.670	2881706	253005
4	Lamivudine	3.668	2883219	248613
5	Lamivudine	3.665	2820008	258215
Mean			2257206	
Std.Dev.			30219.4	
%RSD			1.05	

Table 11: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Emtricitabine	1884	56336	0.110
Lamivudine	43389	47688	3.0

Table 12: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Emtricitabine	1884	56336	0.33
Lamivudine	43389	47688	9.09

Table 13: Showing robustness(flow rate) results for Emtricitabine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing

1	0.8	3696	1.8
2	**1.0**	**3646**	**1.4**
3	1.2	3657	1.8

Table 14: Showing robustness(flow rate) results for Lamivudine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	3108	1.8
2	**1.0**	**3348**	**1.4**
3	1.2	3057	1.9

Table 15: Showing robustness (organic composition) results for Emtricitabine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	3706	1.75
2	***Actual**	**3646**	**1.4**
3	5 % more	3627	1.8

Table 16: Showing robustness (organic composition) results for Lamivudine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	3309	1.86
2	***Actual**	**3348**	**1.4**
3	5 % more	3220	1.9

4. Conclusion

The RP-HPLC method developed and validated allows a simple and rapid quantitative determination of Emtricitabine and Lamivudine in pharmaceutical dosage forms. All the validation parameters were found to be within the limits according to ICH guidelines.

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