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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LAMIVUDINE, ABACAVIR AND ZIDOVUDINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Developing a single analytical method for estimation of individual drug from a multidrug composition is a very challenging task. A simple, rapid, precise, and reliable reverse phase HPLC method was developed for the separation and estimation of three drugs Lamivudine, Abacavir And Zidovudine in bulk drug mix and pharmaceutical dosage forms. The estimation was carried out using Inertsil ODS 250mm x 4.6 mm, 5µ. column; mobile phase consisting of Buffer, Acetonitrile and methanol 65:15:20; the flow rate of 1 mL/min and ultraviolet detection at 250 nm. All the three drugs were properly resolved having run time of 2.1 min, 2.4 min and 6.9 min for Lamivudine, Abacavir And Zidovudine, respectively. The method was validated as a final verification of method development with respect to precision, linearity, accuracy, ruggedness, and robustness. The validated method was successfully applied to the commercially available pharmaceutical dosage form, yielding very good and reproducible result.

Key words: Lamivudine, Abacavir, Zidovudine, Reverse phase HPLC.

INTRODUCTION

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics for bacteria, specific antivirals are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development. Abacavir[1] is a carbocyclic synthetic nucleoside analogue and an antiviral agent. Intracellularly, Abacavir is converted by cellular enzymes to the active metabolite carbovir triphosphate, an analogue of deoxyguanosine-5'-triphosphate (dGTP). Carbovir triphosphate inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate dGTP and by its incorporation into viral DNA. Lamivudine[2] is a synthetic nucleoside analogue and is phosphorylate intracellularly to its active 5'-triphosphate metabolite, Lamivudine triphosphate (L-TP).This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination. Zidovudine[3], a structural analog of thymidine, inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate dGTP and by its incorporation into viral DNA.For individual estimation of each drug, several methods are available in the literature [4–7] even there are couple of methods available for estimation of three drugs at a time [8–10]. There are Very limited work has been done for the simultaneous estimation of all the three drugs, namely, Lamivudine, Abacavir ,Zidovudine.

For contributing such a novel cause, through this article, we have tried our best to develop a fast and user-friendly methodology for the simultaneous estimation of Lamivudine, Abacavir, Zidovudine using reverse phase-HPLC method in bulk drug mix and pharmaceutical dosage forms.

MATERIALS AND METHODS

In the present work, efforts have been made for the simultaneous estimation of Lamivudine, Abacavir and Zidovudine and its pharmaceutical dosage forms. Several trials have been made with respect to the mobile phase composition, columns, as well as UV detector's wavelength to develop a suitable and fast method for the analysis of all the three drugs, simultaneously. The ultimate method of analysis has been provided.

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Materials, Reagents, and Chemicals

Samples of Lamivudine, Abacavir and Zidovudine Standards were obtained from Spectrum Labs. Combination drug tablets, Trizivir, used for the experiment was manufactured by LUPIN Pharma . HPLC-grade acetonitrile, tetrahydrofuran, dipotassium orthophosphate, trimethylamine, and ortho phosphoric acid were obtained from Merck, Darmstadt, Germany.

Equipments

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Lamivudine and Abacavir and Zidovudine solutions.

Chromatographic Conditions

The Chromatographic column, Inertsil ODS 250mm x 4.6 mm, 5µ. Column with mobile phase consisting of Buffer, Acetonitrile and methanol 65:15:20 flow rate of 1 mL/min and ultraviolet detection at 250 nm at 25°C.

Preparation of Solutions

Buffer: (0.1% OPA) Accurately transfer 1 ml of ortho phosphoric acid in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degasse to sonicate, add 0.5ml of Triethylamine, finally made up the volume with water.

Standard Preparation: Accurately Weighed and transferred Lamivudine 15 mg , Abacavir 30 mg and Zidovudine 30 mg working Standards into a 10ml clean dry volumetric flask respectively , add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1 ml was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluents.

Sample Preparation:

Ten tablete was taken into a 100ml volumetric flask and made up with diluents and labeled as Sample stock solution. Sample stock solution was filtered by HPLC filters. 1.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluents.

Preparation of Linearity Solutions

Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Lamivudine , Zidovudine and Abacavir are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 37.5ppm, 75ppm, 112.5ppm, 150ppm, 187.5ppm, 225ppm of Lamivudine and 75ppm 150ppm, 225ppm, 300ppm, 375ppm, 450ppm of Abacavir and 75ppm 150ppm, 225ppm, 300ppm, 375ppm, 450ppm of Zidovudine.

Sample Preparation for Accuracy

Standard Preparation: Accurately Weighed and transferred Lamivudine 15 mg , Abacavir 30 mg and Zidovudine 30 mg working Standards into a 10ml clean dry volumetric flask respectively , add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1 ml was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluents.

Sample preparation

50%: One tablete was taken into a 100ml volumetric flask and made up with diluents and labeled as Sample stock solution. Sample stock solution was filtered by HPLC filters. 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluents.

100%: One tablete was taken into a 100ml volumetric flask and made up with diluents and labeled as Sample stock solution. Sample stock solution was filtered by HPLC filters. 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluents.

150%: One tablete was taken into a 100ml volumetric flask and made up with diluents and labeled as Sample stock solution. Sample stock solution was filtered by HPLC filters.

Analytical Method Validation

Specificity of the Method

The terms selectivity and specificity are often used interchangeably. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. This parameter was performed to know the retention time of each drug in a mixture and in the sample to understand if any drug-drug interaction or drug-excipient interaction is present shown in Fig no. 1.

System Suitability

System suitability test is used to verify that the resolution and reproducibility of the chromatographic systems are adequate for the analysis to be done. The tests are based on the fact that the equipment, electronics, samples to be analyzed constitute an integral system that can be evaluated as such. The limits for system suitability were set for theoretical plates, resolution, and asymmetry shown in Table no.1.

Linearity

Six concentrations of the standard mixture were injected and chromatogram was recorded. A graph was

plotted for the concentration of the corresponding drug versus area. The correlation coefficient for each drug was calculated and shown in Fig no.2, 3 & 4and Table no.2.

Accuracy

To determine the accuracy in sample preparation method of standard additions was made for measuring the recovery of the drugs. To the standard solution known concentrations of the drug (50%, 100%and 150%) was added. . The accuracy was expressed as the percentage of the analytes recovery and shown in Fig no. 5 and Table no .3.

Method Precision

It is very important that the method developed should be precise. Six replicates of the sample prepared from the commercial tablets were injected and Assay was calculated to measure the repeatability of retention times and peak area of standard and sample shown in Table no .4.

Robustness

To verify the robustness of the method, the analysis was done under variable flow rates. The flow rate as per the developed method is 1.7 mL/min. This has been purposely changed to 1.5 mL/min and 1.9 mL/min and the chromatogram was obtained shown in Table no .5.

LOD: Limit of ditection was calculated by intercept method and LOD for Lamivudine, Abacavir and Zidovudine were found to be 0.01ppm, 0.13ppm and 0.23ppm respectively.

LOQ: Limit of Quantification was calculated by intercept method and LOQ for Lamivudine, Abacavir and Zidovudine were found to be 0.03ppm, 0.40ppm and 0.70ppm respectively.

Table 1. System suitability parameters of Lamivudine, Zidovudine and Abacavir

Property	Lamivudine	Abacavir	Zidovudine
Retention Time (tR)	2.168± 0.3	2.489± 0.3min	6.993± 0.3min
Theoretical plates (N)	3737± 163.48	5218± 163.48	4966± 163.48
Tailing factor (T)	1.54± 0.117	1.33± 0.117	1.15± 0.117

Table 2. Linearity table of Lamivudine, Zidovudine and Abacavir

S.No	Conc Lamivudine (µg /ml)	Response	Conc Abacavir (µg /ml)	Response	Conc Zidovudine (µg /ml)	Response
1	0	0	0	0	0	0
2	37.5	320752	75	1356135	75	1108162
3	75	640981	150	2614915	150	2100692
4	112.5	960274	225	3900035	225	3283696
5	150	1235788	300	5262557	300	4469909
6	187.5	1593243	375	6588462	375	5504031
7	225	1901937	450	7908749	450	6474816

Table 3. Accuracy results of Lamivudine, Zidovudine and Abacavir

Sample	Amount Added	Amount Recovered	Recovery (%)	% RSD
Lamivudine	75	75.19	100.26	0.72
	150	149.42	99.61	0.44
	225	224.33	99.70	0.24
Abacavir	150	151.1	100.73	1.47
	300	299.03	99.68	0.56
	450	446.81	99.29	1.27
Zidovudine	150	151.33	100.89	1.21
	300	305.37	101.79	0.37
	450	454.32	100.96	1.56

Table 4. Repeatability results of Lamivudine, Zidovudine and Abacavir

Sr.No.	Lamivudine	Abacavir	Zidovudine
1	1290751	5220962	4348344
2	1315548	5205052	4322656
3	1303470	5268317	4290264

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4	1297489	5195557	4295811
5	1309063	5217515	4334452
6	1309208	5201029	4300472
Mean	1304255	5218072	4315333
Std.Dev.	8988.1	26458.0	23406.4
%RSD	0.7	0.5	0.5

Table 5. Robustness data of Lamivudine, Zidovudine and Abacavir

S.NO	Robustness Condition	Lamivudine %RSD	Abacavir %RSD	Zidovudine %RSD
1	Flow Minus	0.2	0.19	0.3
2	Flow Plus	1.27	0.85	1.5
3	Mobile phase minus	0.16	0.25	0.4
4	Mobile phase Plus	0.3	0.3	0.5
5	Temperature minus	0.1	0.2	0.7
6	Temperature Plus	1.27	0.96	1.36

Table 6. Assay of Tablet

S.No.	Lamivudine %Assay	Abacavir %Assay	Zidovudine %Assay
1	98.91	99.95	100.69
2	100.81	99.64	100.09
3	99.89	100.85	99.34
4	99.43	99.46	99.47
5	100.31	99.88	100.37
6	100.33	99.57	99.58
AVG	99.95	99.89	99.92
STD.DEV	0.69	0.51	0.54
%RSD	0.69	0.51	0.54

Table 7. Contents of Summary

Parameters	Lamivudine	Abacavir	Zidovudine
Calibration Range(mcg)	37.5-225ppm	75-450ppm	75-450ppm
Optimized Wavelength	250nm	250nm	250nm
Retention Time	2.168min	2.489min	6.993min
Regression Equation(Y*)	y = 8424.x + 2682	y = 17542x + 277.9	y = 14564x + 321.9
Correlation	0.999	0.999	0
Precision(%RSD)	0.69	0.51	0
% Recovery	99.95%	99.89%	99.92%
Limit of Detection(mcg/ml)	0.01ppm	0.13ppm	0.23ppm
Limit of Quantitation (mcg/ml)	0.03ppm	0.40ppm	0.70ppm

Figure 1. Specificity of Lamivudine, Zidovudine and Abacavir

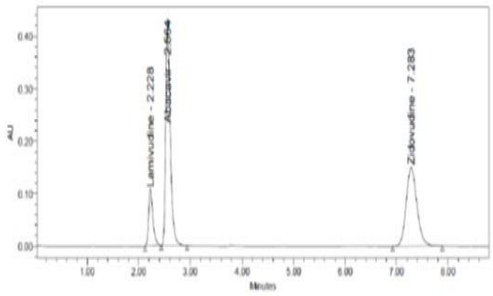

Figure 2. Calibrated curve of Lamuvidine

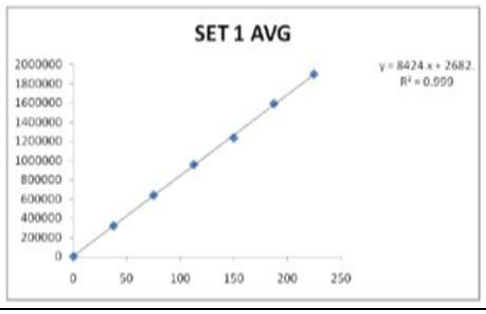

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Figure 3. Calibrated curve of Abacavir

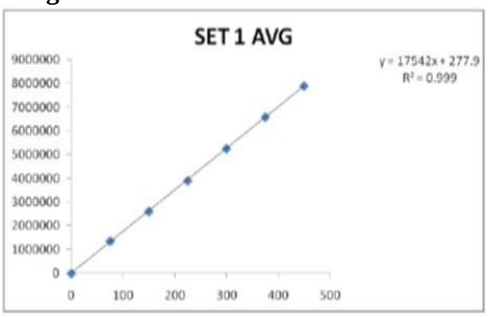


Figure 4.Calibrated curve of Abacavir

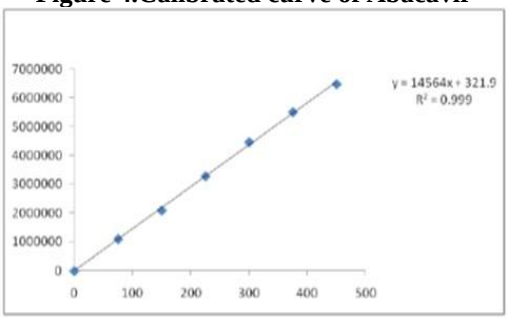
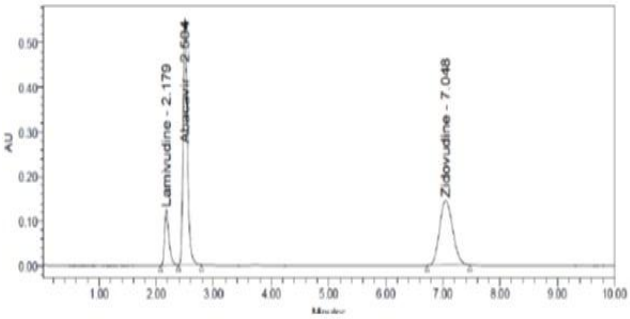


Figure 5. Accuracy 50% of Lamivudine, Zidovudine and Abacavir



RESULTS AND DISCUSSION

After several permutation and combinations, above method has been optimized. It is evident from this method that this is a very fast method of analysis compared to the literature available. We have been able to elute all the three drugs within 7 min. In the current days, industries are looking for the methodology which can save sophisticated instruments and chemist's valuable time, and as a result they can release their product analysis report within lesser time. In this regard, the current method developed by us is very fast and encouraging. The developed method was validated with a holistic approach according to ICH guidelines and details of findings are as below.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Lamivudine, Abacavir and Zidovudine in tablet dosage form. Retention time of Lamivudine, Abacavir and Zidovudine were found to be 2.168min, 2.489min and 6.993min respectively. %RSD of the Lamivudine, Abacavir and Zidovudine were and found to be 0.69, 0.51 and 0.54 respectively. %Recover was Obtained as 99.95%, 99.89% and 99.92% for Lamivudine, Abacavir and Zidovudine respectively. LOD, LOQ values are obtained from regression equations of Lamivudine (0.01ppm, 0.03ppm), Abacavir (0.13ppm, 0.40ppm) and Zidovudine (0.23ppm, 0.70ppm). Regression equation of Lamivudine is y = 8424.x + 2682.2 and of Abacavir is y = 17542x + 277.9 and Zidovudine is y = 14564x + 321.9. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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