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Research Article

STABILITY INDICATING UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE, ABACAVIR AND DOLUTEGRAVIR FROM ITS TABLET DOSAGE FORM

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ABSTRACT

A novel stability indicating Ultra performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous estimation of Abacavir, Dolutegravir and Lamivudine in the tablet dosage form. Chromatographic separations were carried using Inspire (50 mm x 2.1 mm) 1.8 µm column with a mobile phase composition of phosphate buffer (pH 6) and Acetonitrile in the gradient program have been delivered at a flow rate of 0.3ml/min and the detection was carried out using UV detector at wavelength 242 nm. The retention time for Lamivudine, Abacavir and Dolutegravir were 0.965, 1.528 and 2.186 minutes respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 30-150 µg/ml, 60-300 µg/ml and 5-25µg/ml respectively. The results of study showed that the proposed RP-UPLC method is a simple, accurate, precise, rugged, robust, ultra-fast and reproducible, which may be useful for the routine estimation of Lamivudine, Abacavir and Dolutegravir in pharmaceutical dosage form.

Keywords: Abacavir, Dolutegravir, Lamivudine, RP-UPLC, Simultaneous estimation.

INTRODUCTION

Ultra-Performance Liquid Chromatography (UPLC) system is an innovative technique that brought revolution in high performance liquid chromatography by outperforming conventional HPLC. UPLC decreases sample run times up to a factor of 10, uses up to 95% less solvent and significantly improves productivity in the lab as compared to HPLC. The sub-2-µm hybrid particle chemistry, which offers significant benefits over today's HPLC systems, equipped with standard 5-µm particle chemistries. UPLC achieves the speed by using novel sub two-micron particles that reduce chromatographic run times and improve resolution. UPLC was designed as a total system to leverage both ultra-high pressure and small particle separation attributes that result in uniquely superior performance with significant improvements in resolution, sensitivity and speed. UPLC system allows chromatographers to work at higher efficiencies with a much wider range of linear velocities, flow rates and backpressures. UPLC Photodiode Array (PDA) Detector detects and quantifies lower concentrations of sample analyte and trace impurities with maximum sensitivity. The present study was conducted to quantify Abacavir, Dolutegravir and Lamivudine in pharmaceutical dosage form by using RP-UPLC technique.

Lamivudine (Fig. 1) is a synthetic nucleoside analogue with activity against HIV-1 and HBV[1, 2]. The chemical name of lamivudine is (2R, cis)-4- amino-l-(2-hydroxymethyl-l, 3-oxathiolan-5-yl) - (l H)-pyrimidin-2- one. Lamivudine is the (-) enantiomer of a dideoxy analogue of cytidine. Lamivudine has, also been referred to as (-) 2', 3'- dideoxy, 3'-thiacytidine. It has a molecular formula of C8H11N3O3S and a molecular weight of 229.3. Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/ml in water at 20°C.[3]

Abacavir (Fig. 2) is chemically [(1R)-4-[2-amino-6-(cyclopropylamino) purin-9-yl]-1-cyclopent-2-enyl] methanol. It is a white crystalline powder used as antiretroviral agents, for the treatment of HIV infection. It has an empirical formula of C14H18N6O and molecular weight of 286.3323.

Abacavir belongs to a class of antiretroviral drugs known as nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1)[4].

Dolutegravir (Fig. 3) is chemically (3S,7R)-N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclotetradeca-10,13-diene-13-carboxamide. It is a newly developed human immune deficiency virus (HIV-1) integrase inhibitor by binding to the active site and blocking the strand transfer step to retroviral DNA integration. This is an essential step of the HIV replication cycle and will result in an inhibition of viral activity[5].

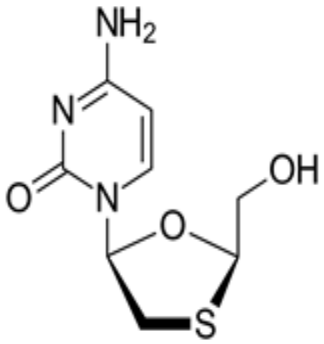

Fig. 1: Chemical structure of Lamivudine

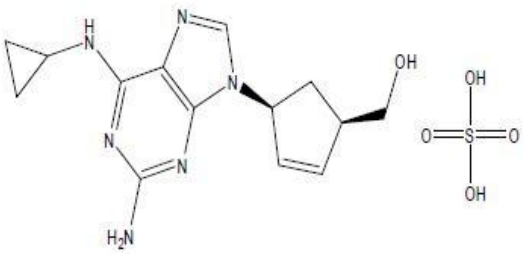

Fig. 2: Chemical structure of Abacavir

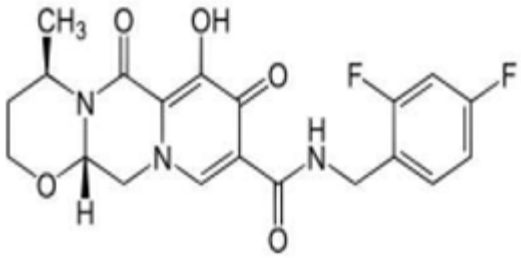

Fig. 3: Chemical structure of Dolutegravir

Various UV[6-9], HPLC and LC[10-14] assay methods were reported in the literature for the estimation of Abacavir, Lamivudine and some other antiretroviral drugs individually and in-combination with other drugs. Hence, we planned to develop and validate a new method for stability-indicating simultaneous determination of Abacavir, Lamivudine and Dolutegravir in bulk drug and pharmaceutical dosage form. The new method is capable of separating all three active analytes present in the dosage form.

System suitability parameters

To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.3ml/min for 3 minutes to equilibrate the column at 40°C temperature. Chromatographic separation was achieved by injecting a volume of 4 µL of standard into Inspire (50 mm x 2.1 mm) 1.8 µm column, the mobile phase of composition phosphate buffer and acetonitrile in the gradient program was allowed to flow through the column at a flow rate of 0.3ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

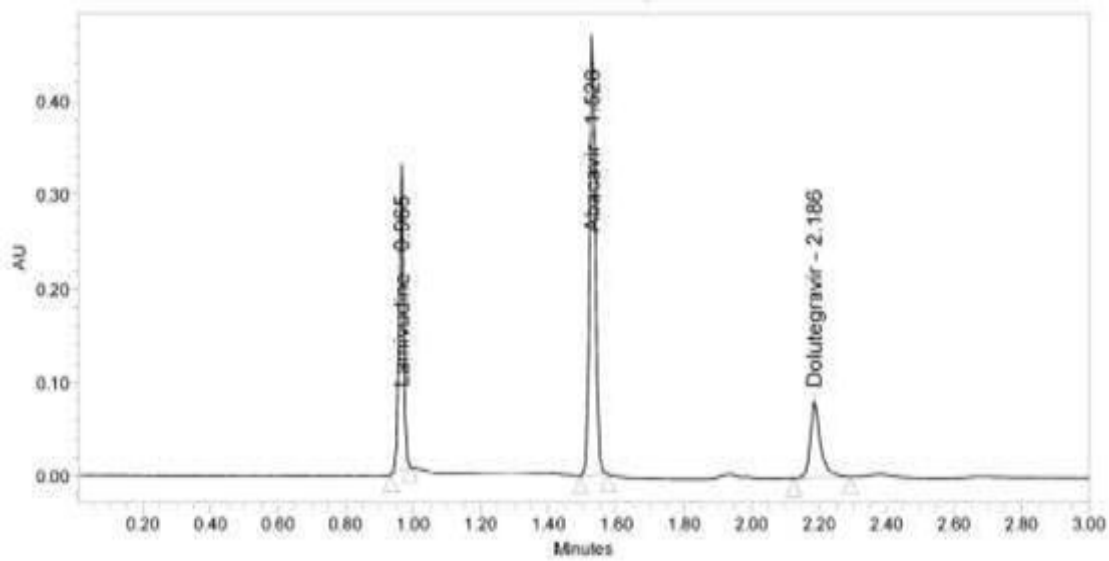

Fig. 4: Standard chromatogram showing separation of Lamivudine, Abacavir and Dolutegravir)

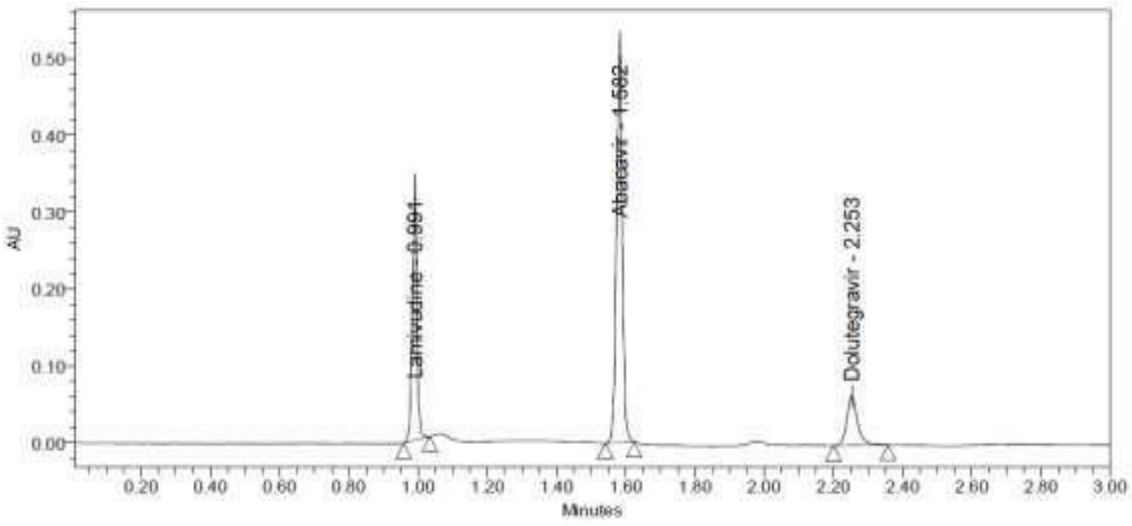

Fig. 5: Sample chromatogram showing separation of Lamivudine, Abacavir and Dolutegravir)

Assay of pharmaceutical formulation

The proposed method was successfully applied to determine Lamivudine, Abacavir and Dolutegravir in their tablet dosage form of brand name TRIUMEQ®. The result obtained for Abacavir, Dolutegravir and Lamivudine was comparable with the corresponding labeled amounts and they were shown in Table-2.

Method validation

The method was validated for the following parameters such as linearity, accuracy, precision, limit of detection and quantification and robustness.

Linearity

Stock solution was prepared by dissolving the appropriate amount of Lamivudine, Abacavir and Dolutergravir in diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 60µg/ml to 300 µg/ml of Abacavir, 5µg/ml to 25 µg/ml of Dolutegravir and 30µg/ml to 150 µg/ml of Lamivudine. Linearity of the method was studied by injecting five concentrations of the drug prepared in the diluent in triplicate into the UPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs (Fig 6,7&8). The correlation coefficients, slopes and *Y*-intercepts of the calibration curve were determined. The results were shown in table 3.

Table 1: System suitability parameters

Parameters	Abacavir	Dolutegravir	Lamivudine
Retention time	1.528	2.186	0.965
USP Plate count	23558.46	24791.22	12940.27
USP Tailing	1.08	1.35	0.90

Table 2: Assay results for Abacavir, Dolutegravir and Lamivudine

Drugs	Label Claim (mg)	Amount Found(mg)	% Assay
Abacavir	600	603.480	100.58
Dolutegravir	50	49.875	99.75
Lamivudine	300	294.930	98.31

Table 3: Linearity results for Abacavir, Dolutegravir and Lamivudine

Parameters	Lamivudine	Abacavir	Dolutegravir
Concentration range(µg/ml)	30-150	60-300	5-25
Correlation coefficient	0.999	0.999	0.999
Intercept	13963	19253	12984
Slope	3415	3340	8538

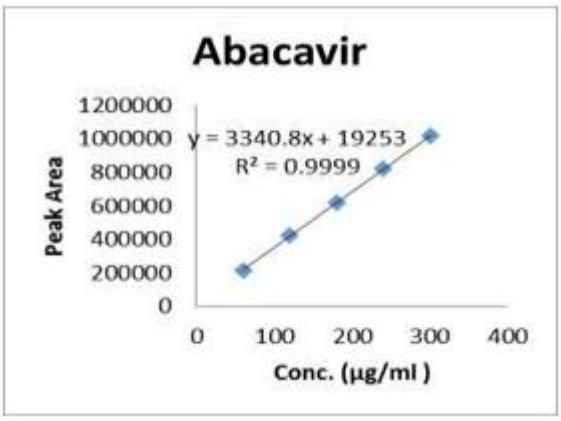

Fig. 6: Linearity graph for Abacavir

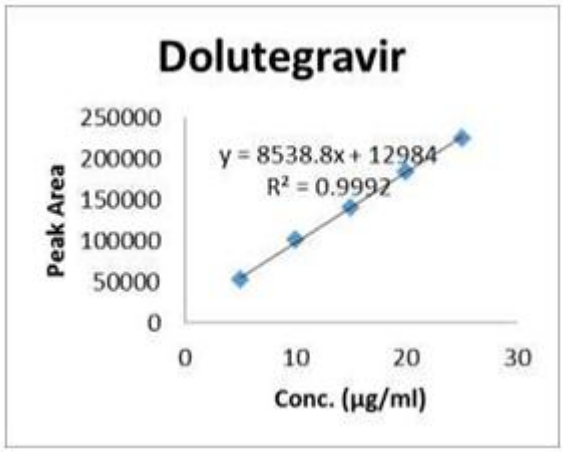

Fig. 7: Linearity graph for Dolutegravir

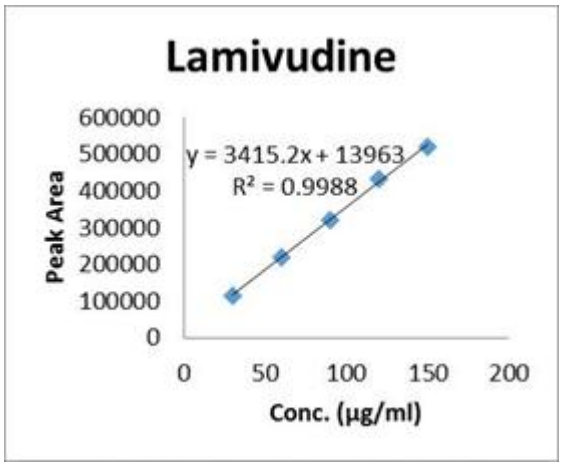

Fig. 8: Linearity graph for Lamivudine

Accuracy

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample. Percent recovery was calculated by comparing the area with preanalysed sample. For all the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by subtracting the total area from preanalysed sample area. The results were shown in Table-4.

Table 4: Accuracy results for Lamivudine, Abacavir and Dolutegravir

Drug Name	Level	Mean Area(n=3)	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
Abacavir	50%	306778	30	29.64	98.79	99.36
	100%	622248	60	60.12	100.19	
	150%	923067	90	89.18	99.09	
Dolutegravir	50%	65543	2.5	2.55	102.07	101.40
	100%	129298	5	5.03	100.68	
	150%	195428	7.5	7.61	101.45	
Lamivudine	50%	165798	15	15.31	102.09	100.68
	100%	327848	30	30.28	100.93	
	150%	482416	45	44.56	99.01	

Precision

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

The standard solution was injected five times and the area of peak was measured for all five injections in HPLC. The % RSD for the areas of peaks of five replicate injections was found to be within the specified limits. The Precision results were shown in Table-5.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was performed on different days. For intermediate precision studies, 6 replicate tablet solutions were injected. %RSD was determined for peak areas of Lamivudine, Abacavir and Dolutegravir. The acceptance limit should not be more than 2% and the results obtained were found to be within acceptance limits. Results were reported in Table 6.

Table 5: Precision results for Lamivudine, Abacavir and Dolutegravir

Injection Number	Lamivudine	Abacavir	Dolutegravir
1	319587	612067	138595
2	319507	615813	136055
3	319430	613182	133216
4	318618	618956	134704
5	319220	612277	132589
Average	319272	614459	135032
Standard deviation	390.45	2922.74	2403.91
% RSD	0.12	0.47	1.78

Table 6: Precision results for Lamivudine, Abacavir and Dolutegravir

Injection Number	Lamivudine	Abacavir	Dolutegravir
1	310429	616775	135086
2	313105	619141	135460
3	311593	611258	136896
4	312573	610228	138185
5	313726	615564	137251
6	311649	613788	135143
Average	312179.2	614459	136337
Standard deviation	1191.02	3379.47	1290.17
% RSD	0.38	0.54	0.94

Robustness
Robustness of the method was determined by small deliberate changes in flow rate and column oven temperature. The content of the drug was not adversely affected by these changes as is evident from the low value of relative standard deviation indicating that the method was robust.

Limit of detection and Limit of quantification
The limit of detection (LOD) and limit of quantification (LOQ) of the method were determined by standard deviation of response and slope method.

Degradation studies
The International Conference on Harmonization (ICH) guidelines entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Exforge using the proposed method.

Acid degradation condition
Pipette 3ml of the above stock solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 80ºC for 1 hour and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Alkali degradation condition
Pipette 3 ml of the above stock solution into a 10ml volumetric flask and add 3 ml of 0.1N NaOH was added in 10 ml of volumetric flask. Then, the volumetric flask was kept at 80ºC for 1 hour and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Thermal induced degradation
The sample was taken in petridish and kept in Hot air oven at 105^0C for 48 hours. Then the sample was taken and diluted with diluents to prepare 180 ug/ml, 15µg/ml and 90 ug/ml of, Abacavir, Dolutegravir and Lamivudine and injected into UPLC and analysed.

Oxidative degradation
Pipette 3 ml of the above stock solution into a 10ml volumetric flask 3 ml of 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent . The volumetric flask was then kept at room temperature for 30 min. Filter the solution with 0.45 microns syringe filters and place in vials.

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