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Development and Validation of RP-HPLC Method for the Estimation of Abacavir, Lamivudine and Zidovudine in Pharmaceutical Dosage Form

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Abstract: A method has been developed and validated for the estimation of abacavir, lamivudine and zidovudine by high performance liquid chromatography (HPLC) on a C_{18} column with UV detection at 270 nm. The mobile phase composition that provides an optimal resolution of components in an acceptable elution time in water: methanol (70: 30 v/v) with 0.1 % potassium dihydrogen phosphate pH 3.2 (adjusted with ortho phosphoric acid). The powdered tablet were extracted with methanol: water (50:50 v/v) mixture and after addition of stavudine, an internal standard subjected to HPLC analysis and assayed by comparison of analyte to internal standard peak areas to concentration ratios. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from the tablet excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

Keywords: Abacavir, Lamivudine, Zidovudine, HPLC determination.

Introduction

Abacavir, lamivudine and zidovudine are synthetic nucleoside analogs showing a potent and synergistic effect on inhibition of the human immunodeficiency virus (HIV-1), the causative agent of acquired immuno-deficiency syndrome[1] (AIDS). HIV encodes at least three enzymes: protease, reverse transcriptase and endonuclease. The abacavir, lamivudine and zidovudine belong to the class of nucleoside reverse transcriptase inhibitors (NRTI). New therapeutic strategy of AIDS treatment requires the combination of these antiretroviral (ARV) drugs. The introduction of highly effective combination regimens of ARV drugs has led to substantial improvements in morbidity and mortality. The formulations contain three nucleoside analogs (abacavir sulfate, lamivudine and zidovudine) and are intended for patients whose regimen would otherwise include these three components. Abacavir is converted by cellular enzymes to the active metabolite, carbovir triphosphate (CBV-TP), an analogue of deoxyguanosine-5'-triphosphate(dGTP).Intracellularly, lamivudine is phosphorylated to its active 5'-triphosphate metabolite, lamivudine triphosphate (3TC-TP). Intracellularly, zidovudine is phosphorylated to its active 5'-triphosphate metabolite, zidovudine triphosphate (ZDV-TP). The chemical names of abacavir sulfate is (1*S,cis*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt) (2:1), lamivudine is (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one and zidovudine is 3′-azido-3′-deoxythymidine[2].

Numerous analytical methods employed like spectrophotometry[3] and liquid chromatography[4-14] have been reported of individual or multi-component combinations assay of NRTI in biological fluids and pharmaceutical dosage forms. The reported LC methods differ with respect to extraction procedure,

the eluent used for RP-HPLC and the UV detection wavelength. The development and validation of simple, rapid, accurate and precise combined assay for abacavir, lamivudine and zidovudine in tablet formulations are now reported in this paper using RP-HPLC with UV detection at 270 nm.

Experimental

Equipment

Chromatographic separations were made on Hypersil C_{18} column of the following characteristics 250 x 4.6 mm I.D., particle size 5 μm and the injected volume was 10 μL and the column was maintained at 298 K. The absorbance was monitored at 270 nm. The mobile phase was water: methanol (70:30 v/v) with potassium dihydrogen phosphate pH 3.2 adjusted with ortho phosphoric acid. Chromatographic analysis was performed on Agilent 1100 HPLC system equipped with a Jasco (Japan) PV-2080 pump was used to deliver the mobile phase to the analytical column, Hypersil C_{18}, 250 x 4.6 mm I.D., 5 μm particle size purchased from Keytech (Japan). Sample injection was performed via a Rheodyne 7725 injection valve (Rheo dyne, USA) with a 20 μL loop. Detection was achieved by an UV-2075A UV-Visible detector (Jasco, Japan). Jasco borowin soft ware, (Japan) was used for quantitative determination at eluted peaks. Degassing of solvents achieved by helium sparging before use. Dissolution of compound was enhanced by sonication on band line Sonerex (Bandeline, Berlin). The pH of the solution was adjusted by using digital pH meter, model DI 707 (Digiruh Electronics, Hyderabad, India).

Reagents and chemicals

Standards of abacavir sulphate (purity, 98.9 %), lamivudin (purity, 99.7 %), zidovudine (purity, 99.4 %), stavudine (purity higher than 99.8 %) were generously supplied by Aurobindo Pharma Ltd (Hyderabad, India) and Trizivur tablets were supplied by Glaxo Wellcome operations (Greenford, UK). Each film coated tablet contains normally 300 mg abacavir as abacavir sulfate (351 mg), 150 mg lamivudine, and 100 mg zidovudine. In active ingredients excipients involve magnesium stearate, micro crystalline cellulose and sodium starch glycollate. The coating film is made of hydroxy propyl methyl cellulose, polyethylene glycol, titanium dioxide and yellow iron oxide. Potassium dihydrogen phosphate and ortho phosphoric acid from Spinco Lab Ltd (Hyderabad, India) are of analytical grade.

Preparation of stock solution

The separate stock solutions of abacavir, lamivudine, zidovudine and stavudine were prepared by accurate weighing of corresponding standards. Approximately 100 mg of standards were accurately weighed, each into a separate 100 mL volumetric flask, 100 mL of diluent (70:30 v/v water : methanol mixture) was added and mixture was sonicated for 15 min (Bandelin Sonorex). The content of abacavir was 1 mg/mL, zidovudine was 1 mg/mL, lamivudine was 1 mg/mL and stavudine was 1 mg/mL.

Preparation of standard solution

Working standard solutions were prepared by dilution of appropriate volume of stock solutions to zidovudine 300 μg/mL, lamivudine 150 μg/mL, and abacavir 300 μg/mL. Internal standard of stavudine was prepared from the stock solutions made the concentration 20 μg/mL, all solutions were stored at -20^{o}C.

Calibration curve

The separate standard calibration lines were constructed for each component. Different volumes of stock solutions were accurately transferred into 10 mL volumetric flask and diluted to mark yield 0.5-400 μg/mL concentration range for each component. The concentration of internal standard was the same in all solutions 20 μg/mL. The calibration line was obtained by plotting the analyte to internal standard peak area ratios against concentration.

Sample preparation

Ten tablets were accurately weighed (to obtain the average mass of one tablet) then finally powdered and 1371 mg (average mass at one tablet) at homogenized powder was transferred to 100 mL volumetric flask. Approximately 80 mL of 50:50 v/v water: methanol diluent was added and the mixture was sonicated for 15 min. The mixture was then diluted to volume with the diluent. The solution was then filtered off through a 0.22 μm nylon filter, discarding the first 10 mL of filtrate. The 10 mL of the filtrate was collected and diluted to 100 mL in a volumetric flask, with the diluent. The solution contained approximately 0.3 mg/mL of abacavir, zidovudine and 0.15 mg/mL lamivudine. Three aliquots: 3 mL, 5 mL, and 7 mL were taken for HPLC analysis and diluted to 10 mL with the diluent (solutions A1, A2 and A3 respectively). To each solution the IS was added so that its concentration was 0.02 mg/mL.

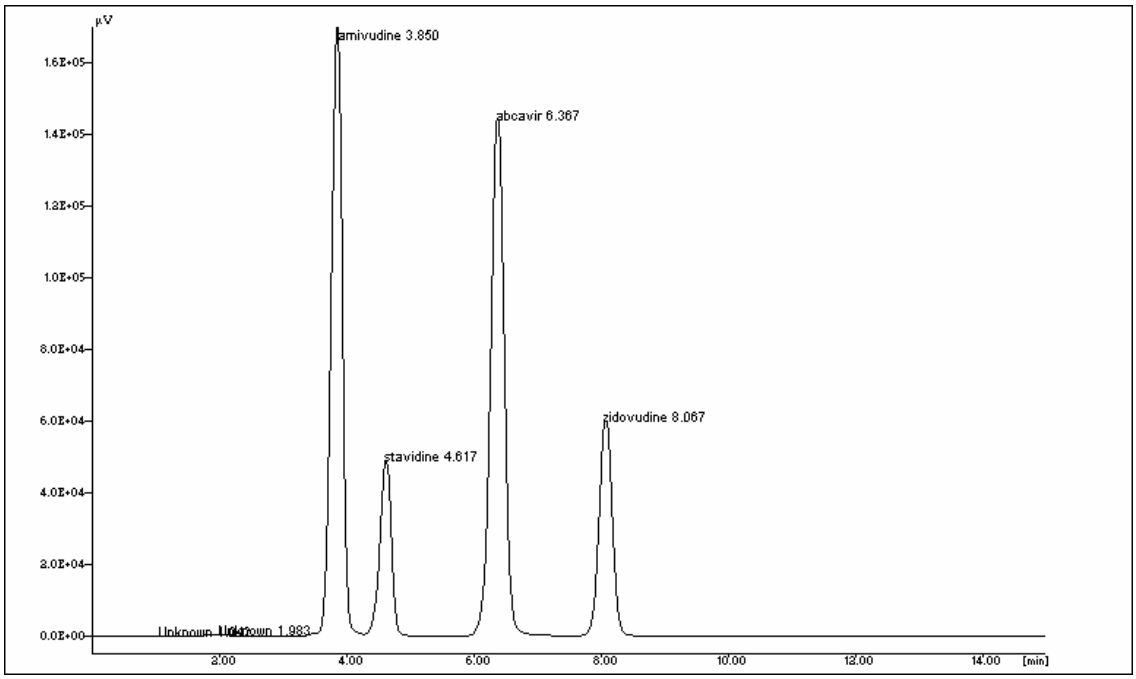

Fig. 1: Typical chromatogram of abacavir, lamivudine, zidovudine and stavudine

Table-1: Accuracy data of proposed method

Analyte	Amount of analyte (µg/ml) taken	Amount of recovery (µg/ml) Mean±S.D.	% of recovery Mean±S.D.
Abacavir sulphate	25	25.27±0.303	101.08±1.200
	50	50.3±0.424	100.6±1.21
	100	100.9±0.629	100.9±1.79
Lamivudine	25	24.61±0.681	98.47±2.7
	50	49.75±0.32	99.5±0.64
	100	99.90±0.518	99.90±0.5
Zidovudine	25	24.85±0.37	99.4±1.51
	50	49.85±0.49	99.66±0.99
	100	101.42±0.42	101.14±0.417

Table-2: Precision data for proposed method

Analyte	Concentration taken (µg/ml)	Intra-day measured concentration (µg/ml)±S.D.	Inter-day measured concentration (µg/ml)±S.D.	% RSD
Abacavir sulphate	25	25.27 ±0.30	25.00 ±0.30	1.99
	50	50.22 ±0.43	50.77 ±047	1.23
	100	100.23 ±0.63	100.52 ±0.62	1.80
Lamivudine	25	24.61±0.681	98.47±2.7	2.11
	50	49.75±0.32	99.5±0.64	2.00
	100	99.90±0.518	99.90±0.5	1.98
Zidovudine	25	24.86 ±0.376	25.03 ±0.42	1.52
	50	49.82 ±0.494	49.7 ±049	0.98
	100	100.4 ±0422	101.2 ±0.422	0.42

Table-3: System suitability parameters

Parameter	Value
Resolution	4.0
Capacity factor	2.47
Theoretical plates	2978
Tailing factor	1.2

Table-4: Limit of detection and limit of quantification

Analyte	LOD (µg/mL)	LOQ (µg/mL)
Abacavir sulphate	0.013	0.044
Lamivudine	0.070	0.230
Zidovudine	0.030	0.102

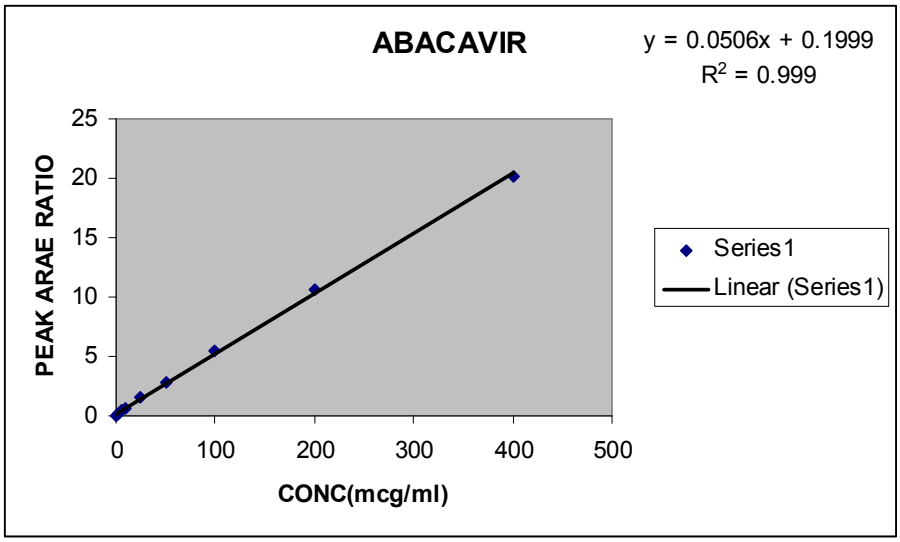

Fig. 2: Linearity curve for abacavir

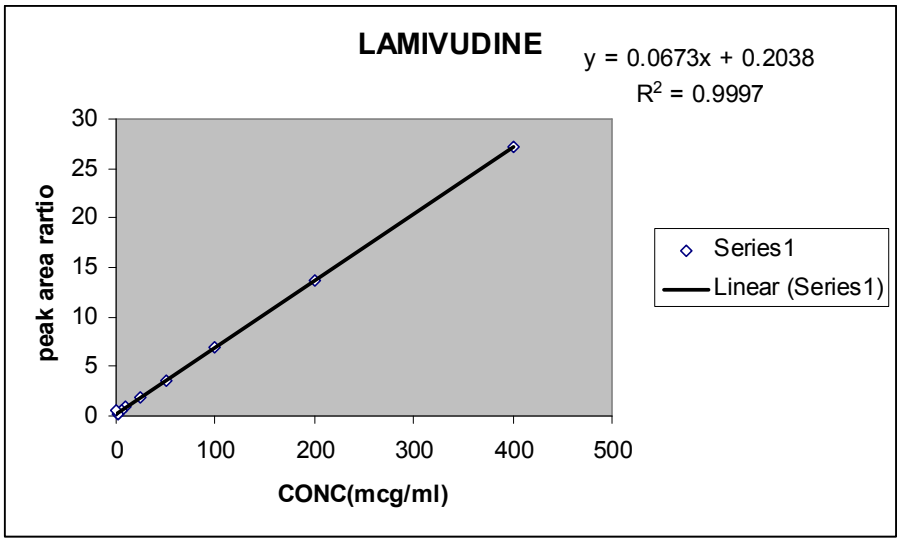

Fig. 3: Linearity curve for lamivudine

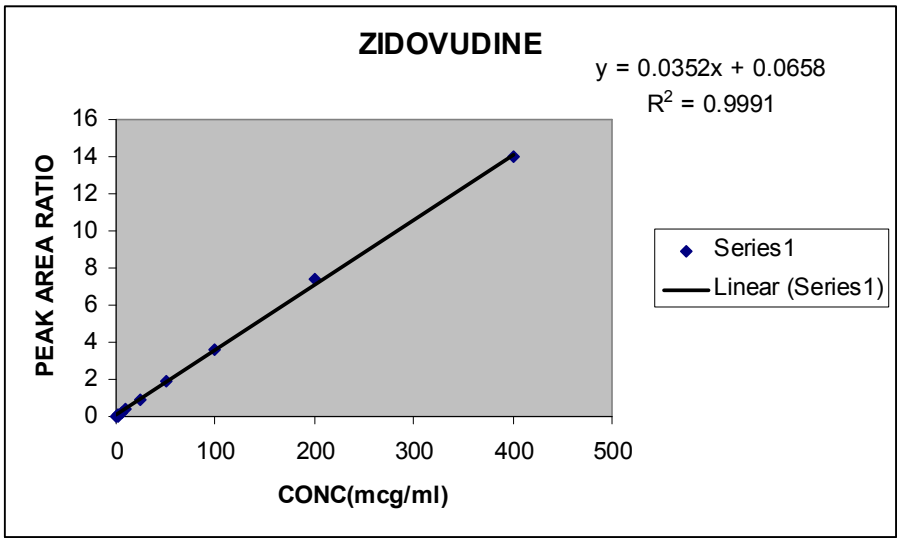

Fig. 4: Linearity curve for zidovudine

Results and Discussion

The mobile phase consisiting of water: methanol (70:30 v/v) with 0.1 % potassium dihydrogen phosphate pH 3.2 (adjusted with ortho phosphoric acid) was selected because it was found to give a base line separation for the peaks of abacavir (Rt=6.3min), lamivudine (Rt=3.8min), zidovudine (Rt=8.6min) and stavudine (Rt=4.6min). Wavelength was selected by scanning standard solution of both drugs over 200 nm to 400 nm. All the compounds show reasonably good response at 270 nm. The methods also applicable to tablet formulation all peaks are shows good separation in Fig. 1.

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and represented in Table-1. The high percentage of recovery indicates that the proposed method is highly accurate.

The precision of the method was demonstrated by inter-day abd intra-day variation studies. Six replicated injections of sample solutions were made and the percentage RSD was calculated and represented in Table-2. From the data obtained the developed RP-HPLC method was found to be precise.

Linearity

The linearity of method was determined at concentration level ranging from 0.5 to 400 µg/mL.

System suitability parameters

As per USP, system suitability tests were carried out on mixed standard stock solutions of abacavir, lamivudine, zidovudine and stavudine. 10 µL of the solution were injected into the chromatographic conditions. Parameters that were studied to evaluate the suitability of system were, number of theoretical plates, resolution and retention time. The values are shown in Table-3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drugs by the proposed HPLC method.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentration of the standard solutions using developed RP-HPLC method. The results were represented in Table-4.

Conclusion

The proposed HPLC method is simple, rapid, sensitive, precise and accurate for the determination of abacavir, lamivudine and zidovudine and can be reliably adopted for routine quality control analysis of bulk drug and its tablet dosage forms.

Acknowledgements

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