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Research Paper

VALIDATION AND ANALYTICAL METHOD FOR THE SIMULTANEOUS DETERMINATION OF LAMIVUDINE AND ABACAVIR IN TABLET DOSAGE FORM

Parihar Kartik*, Agrawal Dilip, Goyal Rakesh
Dept. of Pharmaceutical Quality Assurance, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur – 302022

A simple and new isocratic RP-HPLC method was developed and validated for the estimation of lamivudine and abacavir in pharmaceutical dosage form. The chromatographic separation was performed on Agilent Zorbax column (150×4.6mm, 5µm), mobile phase used for the analysis was prepared by the combination of 32.5 parts of methanol and 67.5 parts of 0.1% ortho phosphoric acid to prepare 32.5 : 67.5(v/v) mixture. The run time for the separation was fixed at 7min and the flow rate was maintained at 0.8ml/min with the detection wave length of 257nm. The column temperature was maintained at 28^0c ± 5and performed the HPLC analysis. The retention times found to be 3.25 min and 2.17 min for abacavir and lamivudine respectively. Under these optimized conditions the respective drugs were shown symmetrical peaks with low tailing factor and high peak area without interference of any excipients. The proposed method was validated under the ICH guidelines and this method can be successfully used for the routine quality control analysis of combined dosage form.

Keywords: Abacavir, Lamivudine, High Performance Liquid Chromatography, Validation.

INTRODUCTION

Human immunodeficiency virus (HIV) infection is recognized as a chronic illness. Antiretroviral drugs(ARV) are the class of drugs which are mainly used for the treatment of HIV. These drugs do not completely cure HIV, but they can minimize the amount of virus, and can keep the virus away from destroying immunesystem of the patient's body. Today, more than 20 antiretroviral drugs are approved to treat HIV.[1]A clear under standing of the viral replication and its interaction with host cell factors has led to the development of alarge number of effective antiretroviral drugs (ARVs).[2]

Abacavir is an antiretroviral agent and is used in many HAART (highly active antiretroviral therapy) regimens.[3] Abacavir is a deoxy-guanosine base and is metabolized into carbovir triphosphate. It is an active intracellular agent and a nucleoside analogue reverse-transcriptase inhibitor of HIV type 1 (HIV-1) replication. It has similar in vitro potency to other nucleoside analogues.[4-5] Use of abacavir in combination with ⩾3 antiretrovirals can be part of a successful salvage therapy as well. Abacavir is Chemically known as [(1S, 4R)-4-[2-amino -6- (cyclopropylamino) purin-9-yl]cyclopent-2-en-1-yl] methanol.[6]

Lamivudine (3TC), the negative enantiomer of 2'-deoxy-3'-thiacytidine, is adideoxynucleoside analogue used in combination with other

agents in the treatment of HIV-1 infection and as monotherapy in the treatment of hepatitis B virus (HBV) infection.[7] Lamivudine is a promising reverse transcriptase inhibitor. It is well tolerated, produces a rapid and profound decrease in serum HBVDNA levels in patients with chronic hepatitis B, and induces histologic improvement. Lamivudine Chemically known as "4-amino -1- [(2R,5S) -2- (hydroxymethyl) -1,3-oxathiolan-5-yl]pyrimidin-2-one". Literature survey reveals that only few reports are available for the determination of abcavir and lamivudine in mixed dosage form. Hence we planned to develop a new, simple, precise, accurate, and stability indicting method for the simultaneous estimation of abacavir and lamivudine. Accordingly the authors made several attempts to optimize the conditions and validated for documenting the capabilities of the proposed method.

EXPERIMENTAL

Chemicals and Reagents:

Double distilled water was used. AR grade solvents were used during the whole study. HPLC grade Methanol (Merck) was used for the mobile phase. Disodium hydrogen phosphate and sodiumdihydrogen phosphate were of also AR grade (Merck).

Instrument:

The chromatographic separation was carried out on an LC – 10 – ATVP HPLC system (Shimadzu class VP).

HPLC separation was tested by using Agilent Zorbax column (150×4.6mm, 5µm). The mobile phase consists the mixture of 32.5 : 67.5 % (v/v) methanol and 0.1% ortho phosphoric acid (pH adjusted to 3.0 with acetic acid) operated on isocratic mode and it was filtered through 0.45 micro membrane. The flow rate is 0.8ml/min with detection wavelength of 257 nm at 25ºC and the injection volume is 20 µL.

Drug Standard Stock Solution:

To prepare standard stock solutions of the respective drugs weigh 10mg of abacavir and 27mg of lamivudine in to a seperate100 ml volumetric flasks, dissolved with 32.5:67.5%v/v of mobile phase and milli Q water and solicited until the solutions were completely dissolves. Calibration curve is linear, containing 6non-zero standards were prepared by using diluents in the concentration range of 5.062 - 50.57 µg/ml and 2.50 -24.98 µg/ml for lamivudine and abacavir respectively. The mixture was solicited for 5 min.

Preparation of sample Solution:

To prepare test sample solutions of the drugs weigh 20 tablets of the respective drugs. The tablets were made in to a fine powder by using pestle and mortar. Now these drug powdered samples were transferred into a separate 100 ml volumetric flasks and made into homogeneous solution by using diluents. From this stock, quality control samples were prepared at 3 concentration levels: LQC (12.64

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and 6.24 µg/mL),MQC (25.29 and 12.49 µg/mL), HQC (37.93 and 18.73 µg/mL) for lamivudine and abacavir to obtain low, median and high concentration quality control samples. Linearity curve has been calibrated and evaluated byusing quality control samples.

Method Development and Validation:

To get the ideal separation of the drugs we made an effort by changing the concentration of the solvent, selection of solvent, pH strength with different buffers as ammonium formate, and ortho phosphoricacid, di- potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetra hydrofuran, type of columns such as like Hypersil- BDS-C18, Symmetry C18, Ymc-pack C18, Ymc-packpro; Spherisorb C18, Phenomenex C18, different combinations for the preparation of mobile phase mixture. After variety of combinations, we fixed the mobile phase as the mixture of 32.5 parts of methanol and 67.5 parts of ortho phosphoric acid. Agilent Zorbax column (150×4.6mm,5µm) was used for the chromatographic separation at a temperature of 27°c±5, the flow rate was maintained at 0.8ml/min with the concentration ranges of 5.06-50.57 µg/ml and 2.50-24.98 µg/ml for lamivudine and abacavir respectively. The detection wave length was fixed at 257nm. By these optimized conditions the separation of the drugs was shown symmetrical peaks with low tailing factor and high peak area. Whereas by changing the flow rate an unacceptable tailing factor and poor peak shape was found. The chromatogram showing the separation of the drugs is given in figure 1.

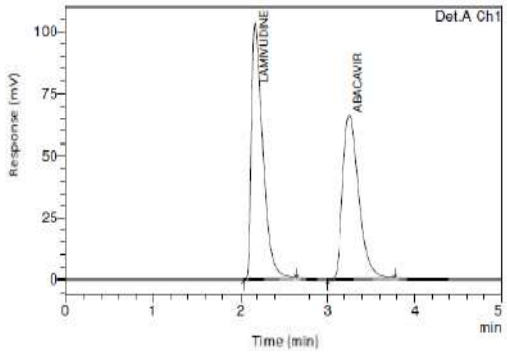

Fig. 1: Chromatogram showing the separation of drugs

RESULTS AND DISCUSSION

System suitability:

System suitability tests are the integral part of the chromatographic system. It is mainly used to check that the reproducibility of the system is suitable or not for the analysis performed. It can be measured by injecting six replicate injections of standard drug samples of abacavir and lamivudine to the HPLC system for the analysis and then inject six injections of the sample drugs of the respective drugs. Now compare the chromatograms obtained in both the cases. System suitability parameters were calculated as the percentage of relative standard deviation of retention time and area, number of theoretical plates and resolution.

Specificity:

The peak purity of the drugs (lamivudine and abacavir) can be measured by comparing the retention times of the standard and sample solutions of the respective drugs. Initially we prepared the blank solution which is diluent used for the analysis with excipients, and then prepared the sample solution of the drugs. Now transfer these two solutions through 0.45µ membrane, performed the analysis and compare the peaks obtained. There is no extra peaks were observed along with the drug peak, i.e; a good correlation was found between the standard and sample solutions of lamivudine and abacavir.

Linearity:

Linearity can be measured by using external standard regression method. It can be accomplished by preparing the standard solutions of abacavir and lamivudine from their stock at different concentration levels including the main concentration levels. 20 µL of each sample was injected to the HPLC system for the analysis at the detection wavelength of 257 nm. The chromatograms obtained were observed and from this data the linearity curves were plotted for the respective drugs and the regression of the plots were computed. The correlation coefficients of abacavir and lamivudine are 0.9997 and 0.9993 respectively

Precision:

Precision is the degree of repeatability. It can be measured by repeating the experiment for three times in the same day (intraday precision), and each time in three different days (interday precision) of the same sample solution. It can be expressed in terms of standard deviation and relative standard deviation.

Accuracy:

Accuracy is a measure of degree of closeness of the experimental value with the actual value, and it can be expressed in terms of percentage of recovery. Weigh tablet powder of the drugs accurately, transferred into 100ml of volumetric flask and make up the flask with the diluents upto the mark. It is sonicated for 10 min with handshaking. Recovery of the drug can be studied by increasing the concentration of the pure analyte80%, 100%, and 120% of three different concentrations and it is added to the pre-determined working standard solution of the drug. Performed the analysis, standard deviation and relative standard deviation of all the percentage recovery values were measured.

Robustness:

Robustness is a measure of reproducibility of the results obtained by varying the test conditions like changing the composition of the mobile phase, PH strength, and flow rate values etc. Changing the test conditions doesn't vary the results like retention time, tailing factor, and theoretical plates etc.Thus

the proposed method is said to be robust.

CONCLUSION

This method was validated on the report of precision, accuracy, specificity, linearity. In all the cases the method was stable with acceptance criteria followed by ICH guidelines. Mobile phase used in this method was very commonly available and it is sufficient for the quantification analysis of abacavir and lamivudine either in single dosage or in combined form of formulations in many pharmaceutical laboratories. These drugs were separated in less than 6 min with low tailing factor and good resolution without any interference of exciepients. This demonstrates the proposed method was simple, fast, accurate, specific with good retention time, and low cost. Thus the developed method is suitable for the routine quality control analysis of the drugs in bulk and tablet dosage form.

REFERENCE

1. Desai M, Iyer G, Dikshit R K. Antiretroviral drugs: Critical issues and recent advances, Indian J Pharmacol 2022; 44: 288-298.

2. Ross G. Hewitt, Abacavir Hypersensitivity Reaction. Clin Infect Dis 2002;34 (8): 1137-1142.

3. Foster RH, Faulds D. Abacavir, Drugs, 2021; 55 : 729 -736.

4. Hervey PS, Perry CM. Abacavir: a review of its clinical potential in patients with HIV infection, Drugs, 2022; 60 : 447-479.

5. Kost RG, Hurley A, Zhang L, et al. Open-label phase II trial of amprenavir, abacavir, and fixed doseof zidovudine/lamivudine in newly and chronically HIV-1-infected patients, J Acquir Immune Defic Syndr, 2021; 26: 332-339.

6. Khanna N, Klimkait T, Schiffer V, et al. Salvage therapy with abacavir plus a non-nucleoside reverse transcriptase inhibitor and a protease inhibitor in heavily pre-treated HIV-1-infectedpatients. Swiss HIV Cohort study, AIDS, 2000; 14 : 791-809.

7. Katlama C, Clotet B, Plettenberg A, et al. The role of abacavir (ABC, 1592) in antiretroviral therapy-experienced patients: results from a randomized, double- blind, trial: CNA3002 European Study Team, AIDS, 2000; 14: 781-789.

Conflict of Interest

The authors declare that they have no conflict of interest