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Original Research Article

SIMULTANEOUS ESTIMATION OF ABACAVIR, LAMIVUDINE AND DIDANOSINE TRIPHOSPHATE IN TABLET DOSAGE FORMULATION BY REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract: A new, simple, rapid, selective, precise and accurate isocratic reversed-phase high performance liquid chromatography assay has been developed for simultaneous estimation of Abacavir Sulphate, Lamivudine and Didnosine Triphosphate in a marketed tablet formulation. The separation was achieved by using C-18 column (Phenomenex, 250×4.6mm *i.d.*) particle size 5µ coupled with a guard column of same material, in mobile phase methanol: Acetonitrile: Ortho phosphoric acid in ratio of 75:25:0.2 respectively with maintain of pH 4.5±0.1 at 1.0 mL/min. Selected wavelength at 245 nm and sample volume injected 20 µL at ambient temperature. Total run time was 10 min; abacavirsulphate, lamivudine and didanosine triphosphate were eluted with retention time of 2.25, 3.55 and 5.50 minutes respectively. The developed method was validated as per ICH guidelines. Validation revealed that method is specific, rapid, accurate, precise, reliable and reproducible.

Key-Word: Method Development, Validation, HIV, Anti-retroviral, Simultaneous estimation.

Introduction: Antiretroviral drugs like nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors are essential in management of HIV infection. The synthetic nucleoside reverse transcriptase inhibitor analogues abacavirsulphate, lamivudine and didanosine triphosphate from one of the fixed dosage combinations used in the effective management of HIV.

Abacavir **(1)** [(1R)-4-[2-amino-6-(cyclopropyl amino)-9H-purin-9-yl}-2-cyclopentene]-1-methanol, is a carbocyclic synthetic analogue. It is rapidly absorbed after oral administration with a bioavailability of about 80%[1].Lamivudine (ZDV) **(2)** is an antiretroviral medication used to prevent and treat HIV/AIDS. It is generally recommended for use with other antiretroviral drugs. It may be used to prevent mother-to-child spread during birth or after a needle stick injury or other potential exposure[2-3]. Didnosine Triphosphate **(3)** was first approved for use in the United States in 1995. It is on the World

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Health Organization's list of essential medications, the most effective and safe medicines needed in a health system[4-7].

Several assay techniques have been described for quantitative determination of Abacabir, Lamivudine and Didanosine Triphosphate in individual and in combination. The UV Spectroscopy determination[8-11], UV and HPLC determination[12-15], HPLC determination[16-20], HPTLC determination[21-25].

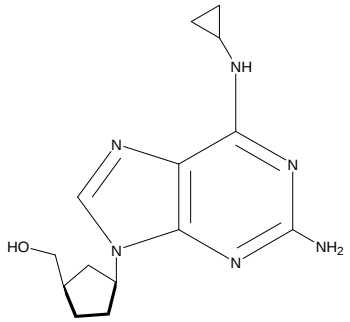

Fig 1 (I) Chemical Structure of Abacavir

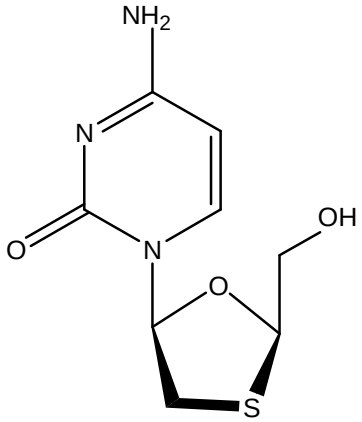

Fig 1 (II) Chemical Structure of Lamivudine

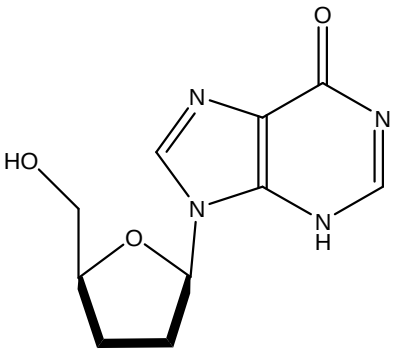

Fig 1 (III) Chemical Structure of Didanosine

Experimental

Material and Methods: Abacavir and Lamivudine were supplied by Zydus Research Center, Ahmedabad, India as gift sample under batch number SWS01566and SWS00976 respectively. Didanosinewas supplied by Cadila Pharmaceuticals, Ahmedabad under batch number 10AS123.All the solvents and reagents used for the analysis were of HPLC grade. While the solvents used for thin layer chromatography were of analytical reagent grade (AR), the HPLC grade water was obtained from Millipore DirectQ3, SSSUTMS, Sehore.

Instrumentation and Chromatographic Conditions: The instrument used was a Shimadzu HPLC system, equipped with a solvent delivery module {LC-10 AT VP}, Rheodyne manual injector 7725i fitted with 20 µL loop and UV detector {SPD-10 A VP}. The separation was achieved on RP C_{18} column {250 x 4.6 mm, 5 micron particle size} using Methanol: Acetonitrile: Ortho phosphoric acid (75: 25: 0.2, v/v/v) as mobile phase. The pH of mobile phase was adjusted to 4.5±0.1 with the help of liquid ammonia. The flow rate was kept at 1 mL/min and the peaks were integrated by UV detector at 230 nm. Operation, data acquisition and analysis were performed using Spinchrom 1.7 software.

Preparation of Stock and Working Standard Solution: Individual stock solutions of the three drugs (600 µg.mL^{-1}) were prepared by dissolving 6 mg of individual drug in 10 ml of mobile phase. The mobile phase standards containing mixture of (I, II, III) were prepared by appropriately diluting the stock in the range of 15-150 µg.mL^{-1} using mobile phase.

Method validation: The method was validated in terms of stability, linearity, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ).

Results and Discussion:

Optimization of Chromatographic Conditions

Spectroscopic analysis of compounds showed that (I) (II) and (III) have maximum UV absorbanceλ_{max} at 212.5, 235 and 240 nm respectively. Therefore, the chromatography

detection was performed at 230 nm using a UV-Visible detector. Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in the mobile phase. Several trails were performed to optimize the mobile phase but satisfactory resolution of drugs could not be achieved. By altering the pH of mobile phase a good separation was achieved. Ortho-phosphoric acid was used as a modifier for better peak shape. The optimized mobile phase was determined as a mixture of Methanol: Acetonitrile: Ortho phosphoric acid (75:25:0.2); pH was adjusted to 4.5 with the help of liquid ammonia. The flow rate was kept at 1.0 mL/min and wavelength was set at 230 nm. Under these conditions (I), (II) and (III) were eluted at 2.25, 3.55 and 5.50 minutes respectively with a run time of 10 min.

A typical chromatogram for simultaneous estimation of (I), (II) and (III) obtained by using the aforementioned mobile phase from 100 µL of the assay preparation is illustrated in Fig 2.

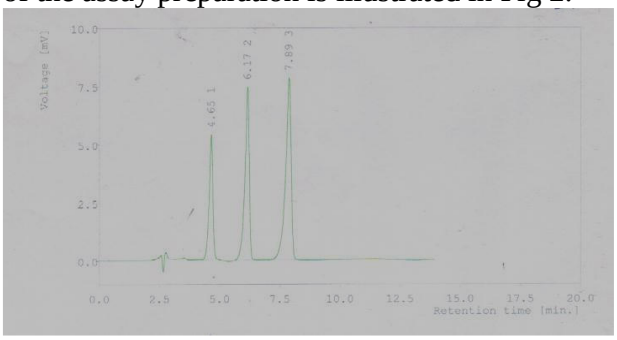

Fig. 2. HPLC chromatogram obtained during simultaneous determination of Abacavir (I), Lamivudine (II), and Didanosine.

Method Validation

Linearity and Calibration standards: The linearity of compounds was evaluated by the analysis of working standard solutions of abacavir, lamivudine and Didanosine of seven different concentrations (0.25-16 µg/mL). Injections of all concentrations (0.25, 0.5, 1, 2, 4, 8 and 16 µg/mL) in triplicate were given and response was recorded for each drug. For peaks that are well resolved, both peak height and area are proportional to the concentration. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients.

All the three drugs were found to be linear in the concentration range of 0.25-16 µg/mL. The coefficient of correlation (r^2) for abacavir, lamivudine and didanosine were found to be 1.00, 0.9999 and 1.00, respectively. The linear equation obtained for abacavir, lamivudine and didanosine were found to be y = 20.249x + 1.3836, y = 18.031x + 0.7946 and 12.744x + 0.3675, respectively.

Specificity: The specificity of the method was determined by comparing the chromatograms of blank solution (mobile phase), solution with tablet excipients (lactose, magnesium stearate, dextrose, carboxymethyl cellulose and talc) and standard drug solution as shown in Fig 2. It was observed that no extraneous peaks were eluted at the same retention time of analytes. Also, the peaks of other two drugs were eluted at different retention time as that of parent drug. The method was thus found to be specific for the analysis of abacavir, lamivudine and didanosine.

Accuracy and Precision: Accuracy of the method was determined by comparing the results of quality control samples with the results of calibration standard curve. The accuracy was calculated as percentage bias and difference ± 2% is often recommended for estimation in bulk and ±20% for estimation in human plasma or biological fluids. The accuracy of the method was found to be in acceptable limit of less than ± 2.0 %. The precision of method was calculated as percentage relative standard deviation (% RSD); which is also known as percentage coefficient of variance (% CV). The % CV less than 5% is acceptable.

System precision for abacavir, lamivudine and didanosine was determined by comparing the standard deviation and percent coefficient of variance (%CV) of retention time, capacity factor, peak asymmetry and resolution of all three concentrations of QC samples of all the three drugs.

LOD and LOQ: The limit of detection (LOD) and limit of quantitation (LOQ) was calculated by the 3:1 and 10:1 ratio of the standard deviation of the peak area of lowest concentration (0.25 µg/mL): slope of the curve, respectively. The limit of detection for (I), (II) and (III) was 0.25 µg.mL^{-1}, 0.45 µg.mL^{-1}, 0.55µg.mL^{-1} respectively and the limit of quantitation (LOQ) was 0.59 µg.mL^{-1}, 065 µg.mL^{-1}, 1.25 µg.mL^{-1} respectively.

Stability studies: The stability of the analyte solutions was determined at intervals of 1st day, 3rd day, and 6th day. The drugs in solution was determined by plotting the standard curve of each drug and mixture on different days; and the results obtained on different days were compared with the results obtained on first day of analysis. In the research work, stability was determined for 14 days. The difference of ±20% in peak area of drug on 1st day versus (v/s) peak area of drug at following days is often acceptable. The data of analysis for stability study revealed that the proposed method was stable for at least 14 days.

Analysis of Tablets: The values of analysis of tablets obtained by the proposed method were between 99.00 % and 101.00 % (Table 1) which showed that the estimation of dosage forms were accurate within the acceptance level of 95 % to 105 %.

Table 1: - Estimation of three drugs in mixture of tablet

Drug	Quantity claimed (mg/tab)	Quantity found (mg/tab)	% quantity found (±SD)
Abacavir	2.0	1.98	98.55 (±0.022)
Lamivudine	2.5	2.51	100.4 (±0.047)
Didanosine	1.0	1.01	100.35 (±0.026)

SD = Standard deviation

Conclusion: An RP-HPLC method for simultaneous estimation of Abacavir, Lamivudine and Didanosine was developed and validated. Results of analysis of the formulations are tabulated in Table 1.The amounts obtained by the proposed method are between 98.55 % to 100.35 %, within the acceptance level of 95% to 105 %. The results obtained indicate that the proposed method is rapid, accurate, selective and reproducible. Linearity was observed over a concentration range of15 to 150 µg.mL^{-1} for all three drugs.

Therefore, it can be concluded that the method has been successfully applied for the analysis of marketed tablets and can be used for the routine analysis of formulations containing any one of the selected drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. The proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations are analyzed by different chromatographic conditions.

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