www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Development and validation method for quantification of Abacavir in formulation and serum analysis by using RP-HPLC

Ramakotaiah M[1]*, Chandrashekar K.B [2], Babu Rao CH[3]

[1]Siddhartha Institute of Pharmaceutical Sciences, Narasaraopet, Guntur (Dt.), A.P, India
[2]Department of Chemistry, Jawaharlal Nehru Technological University, Anatapur, A.P, India
[3]St.Marys College of Pharmacy, Deshmukhi (vill), Nalgonda (Dt), A.P, India

ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Abacavir in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of 0.1%orthophosphoric acid: acetonitrile: methanol 10:25:65 (V/V). The UV detection wavelength was 259nm and 20µl sample was injected. The retention time for Abacavir was 7.005 min. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Abacavir in tablet dosage form and in serum.

Keywords: Abacavir; UV detection; serum; recovery; precise; C18 Column.

INTRODUCTION

Abacavir is a nucleoside analog reverse transcriptase inhibitor used to treat HIV and AIDS. Abacavir Molecular formula $C_{14}H_{18}N_6O$ Molecular weight 286.332g/mol.(Salut M, 2004; Mallal S, 2002) IUPAC Name is (1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-en-1-yl}methanol. Abacavir Literature surveys reveal Spectrophotometeric method atomic absorption spectrometry, spectroflurometry (Veldkamp A.I, 1999; Rauch A, 2006) HPLC (Salut M, 2004) and microbiological method (SFGate.com, 2008) for its determination. Literature survey reveals that Abacavir is estimated by voltametry (Mallal S, 2002) and HPLC (Zucman D, 2007) methods for its determination in dosage forms and biological fluids. It is available under the trade name Ziagen (FDA abacavir alert, 2008; Mallal S, 2008; Mallal S, 2002).

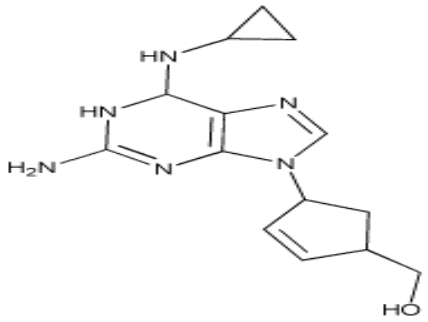

Figure 1: Structure of ABCAVIR

* Corresponding Author
Email: mrk.pharma@gmail.com
Contact: +91-9985909940
Received on: 26-12-2010
Revised on: 15-01-2011
Accepted on: 18-01-2011

EXPERIMENTAL

Chemicals and reagents

Standard bulk drug sampleof Abacavir was provided by Micro Laboratories Ltd., Bangalore. Tablets of combined dosage form were procured from the local market. All other reagents used Actonitrile, Methanol and Orthophosphoric acids were of HPLC grade purchased from Merck Specialties Pvt. Ltd.

Instrumentation and analytical conditions

The analysis of drug was carried out on a PEAK HPLC system equipped with a reverse phase C18 column (250x4.6mm, 5µm in particle size), a LC-P7000 isocratic pump, a 20µl injection loop and a LC-UV7000 absorbance detector and running on PEAK Chromatographic Software version 1.06. Isocratic elution with 0.1%orthophosphoric acid: Acetonitrile: methanol 20:50:30 (V/V) (pH-5.4) was used at a flow rate of 1.0ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

Preparation of Stock, working standard solutions and Sample solutions

Accurately weigh and transfer 10mg of Abacavir working standard into a 10ml volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm nylon filter paper. The calibration curve was plotted with the five concentrations of the 10.0-50.0µg/ml working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

Table 1: Linearity of Abacavir

S.No	Linearity Level	Concentrations in µg/ml	Area
1	I	100	150635.2
2	II	150	231259.5
3	III	200	302296.4
4	IV	250	380054.5
5	V	300	466831.5
Correlation coefficient			0.9998

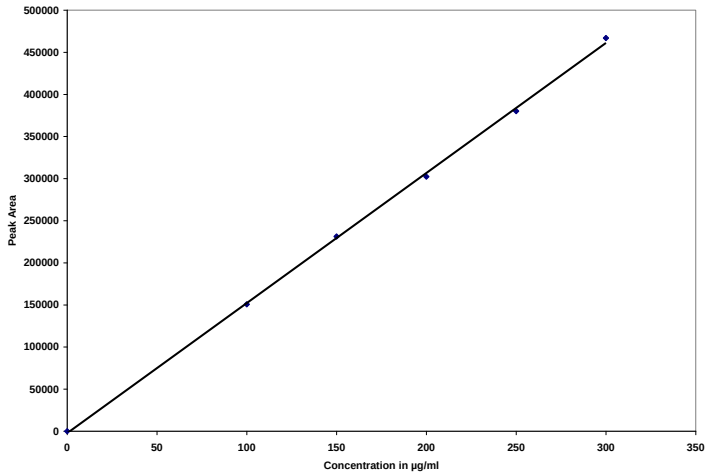

Figure 2: Calibration curve of Abacavir

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 10.0µg/ml to 50.0µg/ml prepared in triplicates to test linearity. The peak area of Abacavir was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from five replicate injections of freshly prepared Abacavir test solution in the same equipment at a concentration value of 100% (100µg/ml) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the Abacavir was determined and precision was reported as %RSD.

Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of Abacavir at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Abacavir recovered in the samples. Sample solution short term stability was tested at ambient temperature (20±10°C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hours at ambient temperature and compared with freshly prepared solutions.

Linearity

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Every 20 µL of the working standard solution of Abacavir in the mass concentration range of 100 to 300 µg/mL, were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Abacavir was obtained by plotting the peak area ratio versus the applied concentrations of Abacavir. The linear correlation coefficient was found to be 0.9998 respectively.

Precision

Repeatability of the method was checked by injecting replicate injections of the solution respectively and the RSD was found to be 0.0318%. Variability of the method was studied by analyzing the solution on the same day (intra-day precision). The results obtained for precision (RSD) is Abacavir.

Table 2: Precision parameters of Abacavir

Concentration in µg/ml	Area	T.plates
1	2077533.9	5508
1	2076940.5	5493
1	2077653.6	5498
1	2078767.4	5478
1	2077818.6	5483
R.S.D		0.0318

Table 3: Accuracy parameters of Abacavir

Abacavir	Concentration in µg/ml	Area	% Recovery
Standard	200	7315.6	100.196%
Sample	200	7330.0	

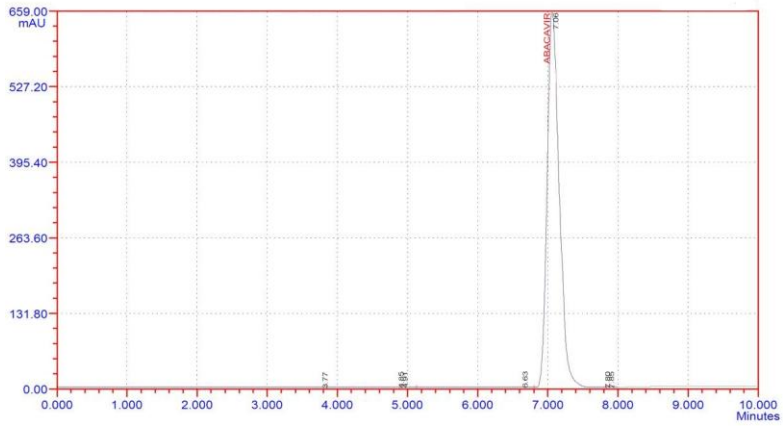

Figure 3: Typical chromatogram of Abacavir Formulation

Accuracy

Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated results are shown in table 3.

Specificity

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 15 µg/ml and 40 µg/ml, respectively as per ICH guidelines.

ESTIMATION OF ABACAVIR IN TABLET DOSAGE FORM

20 Abacavir VIVOL 300 mg tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 3000 µg/ml of Abacavir in to a 10ml volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Abacavir was measured for the determination. The results are furnished in Table 3.

ESTIMATION OF ABACAVIR IN SERUM SAMPLE

PREPARATION OF SERUM SAMPLE SOLUTION

From a Local Government hospital blood was collected and serum was separated. 1ml of this serum was taken in a test tube and 0.1ml of 1M NaOH and 5ml of dichloromethane and mixed about 20min in vortex mixer and centrifuged at 3000 rpm for 10min. From this centrifuged solution 4ml of organic layer was separated and

Table 4: Analysis of Formulation

Formulation	Label claim (mg)	% Amount found
VIVOL	300	99.26

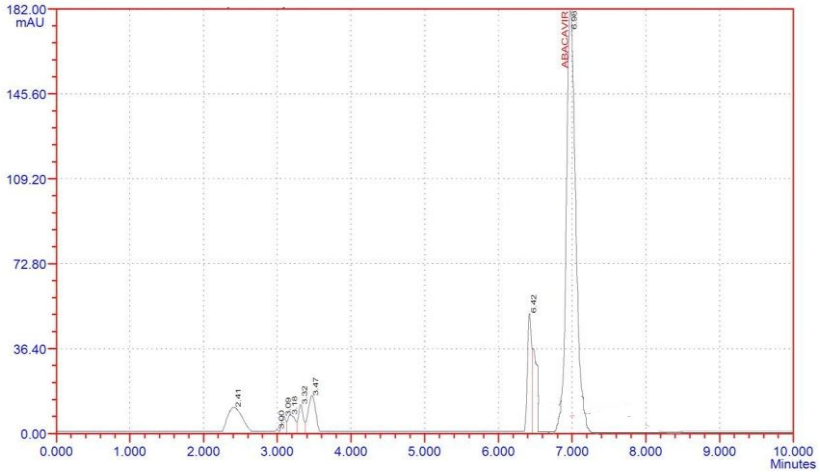

Figure 4: Typical chromatogram of Abacavir in Serum

evaporated to dryness to get residue. To this residue 100µl of 1M Acetic acid and 3ml of n-Hexane and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded. The amount of drug estimated in serum sample is 125.29 µg/ml.

RESULT AND DISCUSSION

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug Abacavir is non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of ortho phosphoric acid, methanol and Acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of Abacavir was thoroughly investigated. The concentration of the ortho phosphoric acid, methanol and Acetonitrile were optimized to give symmetric peak with short run time (Fig.2).

Table 5: Chromatographic conditions of Abacavir

Mobile Phase	0.1% ortho phosphoric acid (10%) Acetonitrile (25%) Methanol (65%)
pH	4.6
UV detection	259nm
Analytical column	C_{18} (250 x 4.6mm)
Flow rate	1.0ml/min
Temperature	Ambient
Injection volume	20µl
Run time	10 min
Retention time	7.005

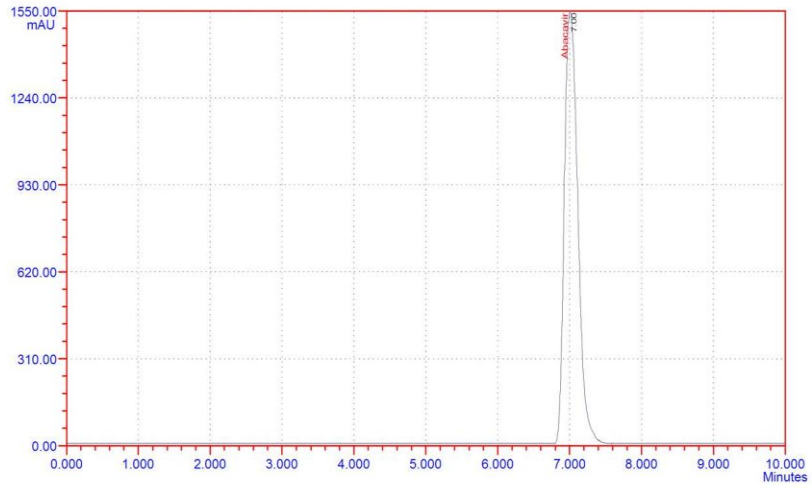

Figure 5: Typical chromatogram of Abacavir

Table 6: System stability parameters of Abacavir

Parameters	Values
λ max (nm)	259
Beer's law limit (µg/ml)	100-300
Correlation coefficient	0.9998
Retention time	7.005
Theoretical plates	5516
Tailing factor	1.84
Limit of detection (ng/ml)	15
Limit of quantification (ng/ml)	40

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Abacavir in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Abacavir in pharmaceutical dosage form.

REFERENCES

Hetherington S, Hughes AR, Mosteller M, *et al.* (2002). "Genetic variations in HLA-B region and hypersensitivity reactions to abacavir". *Lancet* 359 (9312): 1121–2.Doi:10.1016/S0140-6736 (02)08158-8. PMID 11943262.

http://www.fda.gov/cder/drug/InfoSheets/HCP/abacavirHCP.htm FDA abacavir alert web access July 29, 2008.

Mallal S, Nolan D, Witt C, *et al.* (2002). "Association between the presence of *HLA-B 5701, HLA-DR7* and *HLA-DQ3*and hypersensitivity to HIV-1 reverse transcriptase inhibitor abacavir". *Lancet* 359 (9308): 727–32. Doi:10.1016/S0140-6736(02) 07873-X. PMID 11888582.

Mallal S, Phillips E, Carosi G, *et al.* (February 2008). "HLA-B*5701 screening for hypersensitivity to abacavir". *N. Engl. J. Med.* 358 (6): 568–79. Doi: 10.1056/NEJMoa0706135. PMID 18256392.

Veldkamp A.I., Sparidans R.W., Hoetelmans R.M.W., Beijnen J.H. Quantitative determination of abacavir (1592U89), a novel nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection (1999) Journal of Chromatography B: Biomedical Sciences and Applications, 736 (1-2), pp. 123-128.

Rauch A, Nolan D, Martin A, *et al.* (2006). "Prospective genetic screening decreases the incidence of abacavir hypersensitivity reactions in the Western Australian HIV cohort study".*Clin Infect Dis* 43 (1): 99–102. Doi: 10.1086/504874. PMID 16758424.

Salut M. Ferrer,, Pilar Modamio, Cecilia F. Lastra, Eduardo L. Mariño,Article first published online: 6 SEP 2004, DOI: 10.1002/bmc.406,Determination of abacavir in human plasma by high-performance liquid chromatography with ultraviolet detection and the analytical error function

SFGate.com web access July 29, 2008

Zucman D, de Truchis P, Majerholc C, *et al.* (2007). "Prospective Screening for Human Leukocyte Antigen-B Avoids Abacavir Hypersensitivity Reaction in the Ethnically Mixed French HIV Population". *J Acquir Immune Defic Syndr* 45 (1): 1.Doi:10.1097/ QAI. 0b013e318046ea31. PMID17356469.