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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC FOR THE SIMULTANEOUS ESTIMATION OF ABACAVIR SULPHATE AND LAMIVUDINE IN TABLET DOSAGE FORMS

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ABSTRACT

The mechanism of the RP-HPLC is the retention, by the interaction of non-polar hydrocarbon chain of stationary phase with non-polar parts of the sample molecules. This method had been developed for simultaneous determination of antiretroviral drugs which are widely used such as, Abacavir Sulphate and Lamivudine in Tablet dosage form and was carried on column Inertsil ODS (150×4.6, 5µm) with UV detection at 254 nm using a mobile phase composition of mixed phosphate buffer (pH 4.0) and Acetonitrile at a flow rate of 1ml/min. The proposed method was validated in terms of linearity, accuracy, precision, robustness, ruggedness, specificity, limit of detection and limit of quantification as per ICH and USP guidelines and it was found suitable for the routine quality control analysis of the drugs in tablet dosage forms. Linearity of abacavir and lamivudine were found in the range of 20-120µg/ml and 10-60 µg/ml respectively. The limit of detection was found to be 0.0049 and 0.0268 for abacavir and lamivudine respectively. Limit of quantification was found to be 0.0184 µg/ml and 0.0150 µg/ml for abacavir and lamivudine respectively**.** Hence, it was concluded, chromatographic method developed for abacavir sulphate and lamivudine said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

Keywords: Antiretroviral agents, Abacavir Sulphate, Lamivudine, RP-HPLC.

INTRODUCTION

Abacavir and lamivudine 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 and lamivudine 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. Abacavir tablets in combination with other antiretroviral agents, are indicated for the treatment of HIV-1 infection. Abacavir should not be added as a single agent when antiretroviral regimens are changed due to loss of virologic response. Intracellularly, abacavir is converted by cellular enzymes to the active metabolite, carbovir triphosphate [2], an analogue of deoxyguanosine-5' triphosphate (dGTP). Intracellularly, lamivudine is phosphorylated to its active 5'- triphosphate metabolite, lamivudine triphosphate (3TC-TP).

Chemically, abacavir sulfate is (1S,cis)-4-[2-amino-6- (cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1- methanol sulfate , and lamivudine is (2R,cis)-4- amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one [3]. Numerous analytical methods employed for the quantitative determination of individual or multi-component combinations assay of NRTI in pharmaceutical dosage forms. These methods include UV-visible spectrophotometric [4-6] HPTLC [7], HPLC [8-16]. The HPLC method was considered the choice of estimation, since this method is the most powerful of all chromatographic and other separative methods. The reported method differs 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 and lamivudine in tablet formulations are now reported in this paper using RPHPLC with UV detection at 254 nm.

EXPERIMENTAL

Chemicals and Reagents

Standards of Abacavir sulphate (purity 98.9%), Lamivudine (purity 99.7%) were generously supplied by Hetero Drugs Pvt Ltd, Hyderabad. Each film coated tablet contains 600mg Abacavir and 300mg Lamivudine. Inactive ingredients excipients involve magnesium stearate, microcrystalline cellulose and sodium starch glycolate. The coating film is made of hydroxy propyl methyl cellulose, polyethylene glycol, titanium dioxide and yellow iron oxide. Acetonitrile and water are of HPLC grade. All chemicals used were of analytical grade.

Instrumentation

Chromatographic separations were made on Inertsil ODS column of the following characteristics (150 x 4.6)mm I.D., particle size 5µ and the injected volume was 20µL and the column was maintained at ambient temperature. The absorbance was monitored at 254nm. The mobile phase was acetonitrile with mixed phosphate buffer PH 4.0. Chromatographic analysis was performed on Shimadzu Separation Module LC-20AT HPLC. Detection was achieved by a UV detector. Empower 2 software was used for quantitative determination at eluted peaks. Dissolution of compound was enhanced by sonication on ELMa S300H ultra sonicator. The PH of the solution was adjusted by using digital PH meter, Inolab WTW seroes.

Preparation of Stock Solution

117 mg of Abacavir Sulphate (equivalent to 100 mg of Abacavir) and 50mg of Lamivudine working standard/reference standard was accurately weighed and transferred into 100 ml volumetric flask, dissolve with 50 ml of mobile phase, sonicated to dissolve and made up to 100 ml with mobile phase. Shaken well and filtered the solution. From the above filtrate pipette out 5.0 ml into a 50 ml standard volumetric flask and made up to 50 ml with mobile phase.

Preparation of Standard Solution

Working standard solution was prepared by transferring 10ml of the standard stock solution into 50 ml volumetric flask and made up to the mark with diluent and mixed.

Sample preparation

Twenty tablets were accurately weighed (to obtain the average mass of one tablet) then finally powdered and weight equivalent to 100mg of Abacavir and 50mg Lamivudine was weighed and transferred into a 100 ml dried volumetric flask. dissolve with 50 ml of mobile phase , sonicated and made up to 100 ml with mobile phase. The solution was filtered off through a 0.45µ PVDF filter discarding the first few mL. From the above filtrate pipette out 5.0 ml into a 50 ml standard volumetric flask and made up to 50 ml with mobile phase.

Method validation

System Suitability Test

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*R*T), number of theoretical plates (*N*), tailing factor (*T*), and peak asymmetry (*AS*), resolution (RS) were evaluated for five replicate injections of the drug. The system suitability test was performed using five replicate injections of standards before analysis of samples.

Linearity

The calibration curve was constructed for both abacavir and lamivudine. The linearity was evaluated by linear regression analysis, which was calculated by least square method.

Accuracy and Precision

Accuracy of assay method was determined for both intra-day and inter-day variations using triplicate analysis of the QC samples. Precision of the assay was determined by repeatability (intra-

day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the QC samples during the same day.

Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample.

Following optimized conditions were slightly varied.

1) Change in flow rate
2) Change in pH of mobile phase
3) Change in the column temperature, etc.

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions.

Limit of Detection and Quantification

Detection and quantification limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, using the formula

$$\text{Limit of Detection} = \frac{\sigma \times 3.3}{S}$$

$$\text{Limit of Quantification} = \frac{\sigma \times 10}{S}$$

Where,

σ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

RESULTS AND DISCUSSION

System Suitability Test

The % RSD of the peak area and the retention time for both drugs are within the acceptable the range (**Table 1**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 2379 and 2336 and the USP tailing factor was 1.42 and 1.45 for Abacavir and Lamivudine respectively and the resolution between the drugs is 5.958.

Linearity

Peak areas were recorded for each injected concentration of drugs and the calibration curves, concentration v/s peak area were constructed for the drugs. Linearity performance parameters are depicted below. Peak areas were recorded and the graphs, concentration vs. peak area were constructed for the drugs. The statistical data's for both the drugs are tabulated. (**Table 2, Figure 1 and 2**).

Precision

System precision

The results are within acceptance limit, i.e. % RSD was below 2.0 indicating reproducibility of the method shown in the **Table 3.**

Method Precision

The results are within the acceptance limit, i.e. % Drug release was within 95%-105%, indicating reproducibility of the method, shown in **Table 4.**

Accuracy

Accuracy of the method confirmed by studying recovery at 3 different concentrations 50, 100, and 150% of these expected, in accordance with ICH guidelines, by replicate analysis (n=3). Standard drug solution was added to a pre analyzed sample solution and percentage drug content was measured.

$$\%\text{Recovery} = [(c_t - c_u) / c_a] \times 100.$$

Where,

c_t is the total conc. of the analyte found,

c_u is the conc. of the analyte present in formulation;

c_a is the conc. of the pure analyte added to the formulation.

These results indicate the accuracy of the method which are shown in the **Table 5 and 6**.

Robustness

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. The % RSD was found to be below 2% i.e. within the limit. This study signifies that the method is precise under different chromatographic conditions and results are shown in the **Table 7 and 8**.

Ruggedness

Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. It is checked that the results are reproducible under differences in conditions, analysts and instruments. Hence the proposed method was found to be rugged. The results were tabulated.(**Table 9**)

Limit of Detection and Quantification

Detection and quantification limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, by using above given formula and the values are tabulated. (**Table 10**.)

Assay

The results are within the acceptance limits of 95-105% (**Table 11**).

Table 1: System suitability parameters

Parameters	Abacavir	Lamivudine	Acceptance criteria
Area	1704.777	398.070	_
Retention time(min)	2.487	4.107	_
Theoretical plates	2379	2336	NLT 2000
Asymmetry factor	1.42	1.45	NMT 2.0
Resolution(USP)	5.958		NMT 2.0

Table 2: Linearity of Abacavir and Lamivudine

Concentration of Abacavir (µg/ml)	Peak area of Abacavir	Concentration of Lamivudine (µg/ml)	Peak area of Lamivudine
20	356.698	10	84.15
40	686.406	20	166.998
60	976.18	30	232.687
80	1288.844	40	304.665
100	1632.858	50	386.938
120	2012.779	60	462.92

Table 3: System precision of Abacavir and Lamivudine

Injection number(100 mcg/ml)	Rt of Abacavir	Rt of Lamivudine	Area of Abacavir	Area of Lamivudine
Injection-1	2.487	4.107	1704.777	398.070
Injection-2	2.490	4.107	1678.033	396.023
Injection-3	2.493	4.110	1687.676	396.935
Injection-4	2.497	4.117	1674.856	399.940
Injection-5	2.493	4.120	1681.650	396.091
Injection-6	2.492	4.112	1685.398	397.411
Mean	_	_	1685.398	397.412
SD	_	_	10.58451	1.4646
%RSD	_	_	0.63	0.37

Table 4: Method precision of Abacavir and Lamivudine

Injection number(100 mcg/ml)	Rt of Abacavir	Rt of Lamivudine	Area of Abacavir	Area of Lamivudine
Injection-1	2.501	4.118	1692.216	400.325
Injection-2	2.499	4.122	1682.633	401.333
Injection-3	2.507	4.130	1676.333	399.056
Injection-4	2.498	4.128	1689.677	398.730
Injection-5	2.503	4.120	1684.313	399.899
Injection-6	2.507	4.128	1690.650	400.950
Mean	_	_	1685.970	400.048
SD	_	_	6.02345	1.02809
%RSD	_	_	0.36%	0.26%

Table 5: Accuracy of Abacavir

Accuracy Level	Amount Present (mcg/ml)	Amount Added (mcg/ml)	Amount Recovered (mcg/ml)	Percentage Recovery	Mean percentage recovery
80	70	10	79.89	99.8	99.46
80	70	10	79.58	99.4	
80	70	10	79.39	99.2	
100	70	30	99.57	99.5	99.42
100	70	30	99.79	99.2	
100	70	30	99.68	99.6	
120	70	50	119.62	99.6	99.52
120	70	50	119.53	99.6	
120	70	50	119.45	99.4	

Table 6: Accuracy of Lamivudine

Accuracy level	Amount Present (mcg/ml)	Amount Added (mcg/ml)	Amount Recovered (mcg/ml)	Percentage Recovery	Mean percentage recovery
40	35	5	39.76	99.42	99.39
40	35	5	39.69	99.22	
40	35	5	39.82	99.55	
50	35	15	49.65	99.30	99.50
50	35	15	49.85	99.70	
50	35	15	49.76	99.52	
60	35	25	59.74	99.6	99.70
60	35	25	59.96	99.93	
60	35	25	59.75	99.58	

Table 7: Robustness of Abacavir

Proposed variations		Asymmetry factor	AREA	RSD	Acceptance criteria
Variation in Flow Rate	0.9ml	1.387	1489.445	1.3	Asymmetry factor In between 0.5 and 2.0 RSD NMT 2.0
	1.1ml	1.407	1224.394		
Variation in Wavelength	252nm	1.429	1296.228	0.4	
	256nm	1.464	1376.260		

Table 8: Robustness of Lamivudine

Proposed variations		Asymmetry factor	Area	RSD	Acceptance criteria
Variation in Flow Rate	0.9ml	1.500	355.928	1.3	Asymmetry factor In between 0.5 and 2.0 RSD NMT 2.0
	1.1ml	1.442	293.027		
Variation in Wavelength	252nm	1.535	414.465	0.8	
	256nm	1.500	367.903		

Table 9: Ruggedness of Abacavir and Lamivudine

	Retention time of Abacavir	Retention time of Lamivudine	Area of Abacavir	Area of Lamivudine
Analyst(1)(100mcg)	2.493	4.110	1687.676	401.082
Analyst(2)(100mcg)	2.490	4.107	1681.709	404.594
Average	2.491	4.108	1684.69	402.838

Table 10: LOD and LOQ

Sample	LOD	LOQ
Abacavir sulphate	0.0049 µg/ml	0.0184 µg/ml
Lamivudine	0.0268 µg/ml	0.0150 µg/ml

Table 11: Assay of Abacavir and Lamivudine

Abacavir		Lamivudine	
Sample Area	1703.731	Sample Area	405.613
Standard Area	1696.293	Standard Area	395.454
Standard Weight	49.8mg	Standard Weight	24.3mg
Sample Weight	85.2mg	Sample Weight	85.2mg
LC	600mg	LC	300mg
Average Weight	1023.2mg	Average Weight	1023.3mg
Standard Purity	99.85 % w/w	Standard Purity	99.86% w/w
Assay % w/w	99.96 w/w	Assay % w/w	99.65 w/w

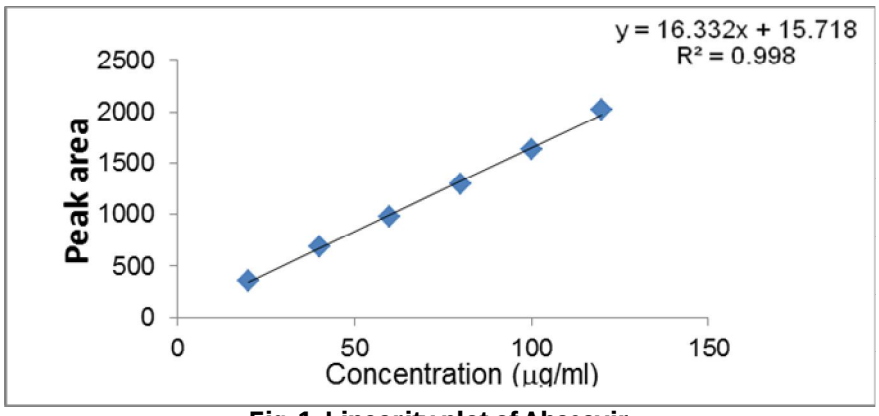

Fig. 1: Linearity plot of Abacavir

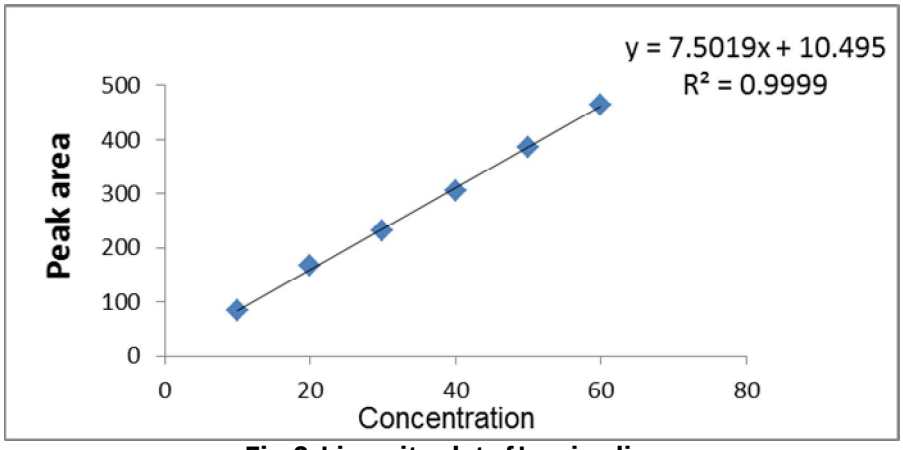

Fig. 2: Linearity plot of Lamivudine

CONCLUSION

From the above experimental data and results, the developed HPLC method is having the following advantages. The standard and sample preparation requires less time.Run time required for recording chromatograms were less than 10 minutes.Suitable for the analysis of raw materials, applicable to dissolution studies and can be used for the content uniformity studies. Hence, the chromatographic method developed for Abacavir sulphate and Lamivudine said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokineticstudies.

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REFERENCES

1. WHO public assessment report, Abacavir Sulfate, Lamivudine and Zidovudine Tablets 300mg/150mg/300mg. 2009;Part 7:1-2.
2. Indian pharmacopoeia. Intracellularly, abacavir is converted by cellular enzymes to the active metabolite, carbovir triphosphate. 1996; 1: A-68-70.
3. www.rxlist.com
4. Srinivas rao M, Ravi kumar D, Vardhan S V M, and D. Ramachandran D., International journal of Chemtech research ., Development of validated spectrophotometric method for the assay of Abacavir sulfate in bulk and pharmaceutical dosage formulations 2011;.3(3), 1200-1202.
5. Venkatamahesh.R and Dhachinamoorthi.D., International Journal of Pharm Tech Research, Visible Spectrophotometric determination of Abacavir Sulphate in Bulk Drug and Tablet Dosage Form 2011, 3(1), 356-359.
6. G.Srihari,N.Rami reddy, and I.E.Chakravarthi. Global Journal of Pharmacology., Spectrophotometric method for the determination of Abacavir sulfate in pharmaceutical preparations 2011; 5(3), 172-175.
7. Sudha, T, Ravikumar.V.R and P.V. Hemalatha, J. Pharm. Sci. and Res.,Validated HPTLC method for simultaneous determination of lamivudine and Abacavir sulphate in tablets dosage form, International 2010; 1(11),101-111.
8. Sparidans WR, Hoetelmans WMR, Beijnen HJ. Journal of Chromatogr-B., Liquid chromatography assay for simultaneous determination of abacavir and mycophenolic acid in human plasma using dual spectrophotometric detection 2001, 750,155-161.
9. Ravitch JR, Moseley GC., Journal of Chromatography- B., High-performance liquid chromatographic assay for acavirand its two major metabolites in human urine and cerebrospinalfluid 2001,762, 165-173.
10. Aymard G, Legrand M, Trichereau N, Diquet B., Journal of Chromatogr - B Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography 2000;744, 227-240.
11. Veldkamp I A, Sparidans WR, Hoetelmans WM R and Beijnen H J.,Journal of Chromatogr B., Quantitative determination of abacavir, a novel nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection 1999;734,123-128.
12. Anantha Kumar D, Srinivasa Rao. G. and Seshagiri Rao JVLN., E-Journal of Chemistry Simultaneous determination of Lamivudine, zidovudine and Abacavir in tablet dosage form by RPHPLC method2010; 7(1),180-184.
13. Summer R. Lewis, Catherine A. White, Michael G. Bartlett., Journal of Chromatography- B., Simultaneous Determination of Abacavir and Zidovudine from rat tissue using HPLC with UV detection 2007: 85, 45-52.
14. Yalcın Ozkan and Ayhan Savaser. Journal of Liquid Chromatography & Related Technologies Simple and reliable HPLC method of Abacavir determination in pharmaceutical, human serum and drug

dissolution studies from tablets 2005;28,423-437.

15. Raja T and Lakshmana Rao. A., International Journal of Pharm Tech Research., Development and Validation of RP-HPLC Method for the Estimation of Abacavir, Lamivudine and Zidovudine in pharmaceutical disage form 2011; 3 (2),852-857.
16. Sudha, T, Ravi kumar V.R and Hemalatha P.V., Journal on Pharmaceutical Biomedical Res., RP-HPLC method for simultaneous Estimation of Lamivudine and Abacavir sulfate in Tablet form. International 2008; 1(4); 108-113.