INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.9.3.2019.950

A STABILITY INDICATING RP-HPLC METODS FOR SIMULTANEOUS ESTIMATION OF THE ABACAVIR, LAMIVUDINE AND DOLUTEGRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

Srinivas Lella*, P. Venkateswararao and N. Srinivasrao

Department of Pharmaceutical Analysis and Quality Assurance,
Vikas College of Pharmacy, Vissannapeta-521 215, Nuzvid,
Tirvur, Andhra Pradesh, India.

ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Abacavir, Lamivudine and Dolutegravir in solid dosage form. Chromatogram was run through Discovery C18 150x4.6mm, 5µ. Mobile phase containing Buffer and Acetonitrie in the ratio of 65:35 v/v was pumped through column at a flow rate of 0.8ml/min. Buffer used in this method was 0.01N KH2PO4 buffer at pH 4.5. Temperature was maintained at 30°C Optimized wavelength for Abacavir. Lamivudine and Dolutegravir was 225.0nm. Retention time of Abacavir, Lamivudine and Dolutegravir were found to be 2.338min, 3.275min and 3.870min %RSD of system precision for Abacavir, Lamivudine and Dolutegravir. were and found to be 0.4, 0.4and 0.4 respectively.% RSD of method precision for Abacavir, Lamivudine and Dolutegravir.

Keywords: Abacavir. Lamivudine, Dolutegravir and RP-HPLC.

INTRODUCTION

Abacavir

Abacavir (ABC) is a powerful nucleoside analog reverse transcriptase inhibitor (NRTI) used to treat HIV and AIDS. Chemically, it is a synthetic carbocyclic nucleoside and is the enantiomer with 1S, 4R absolute configuration on the cyclopentene ring. In vivo, Abacavir sulphate dissociates to its free base. IUPAC Name[1,2,3.]:[(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]cyclopent-2-en-1-yl]methanol,(figure1).

Lamivudine

A reverse transcriptase inhibitor and zalcitabine analog in which a sulphur atom replaces the 3' carbon of the pentose ring. It is used to treat Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV). Chemical name[1,2,3.]: 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.(figure 2).

Dolutegravir

Dolutegravir is indicated for HIV-1 infection for adults and children and adolescents ≥12 years of age and weighing ≥40 kg. It is marketed as Tivicay as Dolutegravir sodium. 52.6 mg of Dolutegravir sodium is equivalent to 50 mg Dolutegravir free acid. FDA approved on August 12, 2013. Chemical name[1,2,3]:(3S,7R)-N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8diazatricyclo [8.4.0.0] tetradeca-10,13-diene-13-carboxamide,(figure 3).

Analytical methods play a vital role in new drug development, preformulation and formulation studies, stability studies, quality control testing and quality assurance programmes. So analysts are always in search of developing rapid and accurate new methods of analysis that are able to exist in routine analytical work. The review of literature reveals that only few chromatographic methods have been reported for the estimation of Abacavir Lamivudine and Dolutegravir like LCMS, LCNMR MS, HPLC[4-6].

There is a need for developing newer methods in HPLC for developing a simple and economic method and so we proceeded with HPLC and validated as per the ICH guidelines. The present analytical work comprises of simple, precise, rapid, sensitive and accurate methods for the estimation of Abacavir Lamivudine and Dolutegravir bulk and dosage form.

EXPERIMENTAL

Instrumental specification Waters HPLC 2695 series with quaternary pumps, Photo Diode array detector and auto sampler integrated with empower software column used was C18 150 x 4.6 mm

MATERIALS AND METHODS

Abacavir Lamivudine and Dolutegravir working Standard was obtained from Spectrum pharma research solutions pvt. Ltd, Hyderabad and commercial dosage forms containing the studied drug were obtained from local market. All the reagents used were analytical reagents, solvents were of HPLC grade

Buffer Preparation

0.1%OPA Buffer

1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

0.01N KH_2PO_4 Buffer

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 4.5 with dil. Orthophosphoric acid solution

Mobile Phase

Buffer and Acetonitirile were mixed in the ratio of 65:35 V/V and sonicated to degas.

Preparation of Standard stock solutions: Accurately weighed 75mg of Abacavir, 37.5 mg of Lamivudine and 6.25mg of Dolutegravir and transferred to three 25ml volumetric flasks separately. 10ml of Diluent was added to flasks and sonicated for 20mins. Flasks were made up with water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with Water methanol.

Preparation of Sample stock solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered.

Preparation of Sample working solutions (100% solution)

From the filtered solution 0.5ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents. (300ppm, 150ppm & 25ppm).

RESULTS

METHOD VALIDATION

The described method has been validated for the assay of Abacavir Lamivudine and dolutegravir using following parameter[7-10].

System suitability

The system suitability parameters were determined by preparing standard solutions of Abacavir, Lamivudine and Dolutegravir and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%. The results were tabulated in Table 1.

Linearity

The linearity of an analytical method is to elite test results that are directly, of by well-defined mathematical transformation, proportional to the concentration of analyse in sample within a given working range. The results were tabulated in Table 2.

System Precision

Prepared and analyzed six replicate sample preparations as per method. And calculate the %RSD of area of response. The results were tabulated in Table 3.

Repeatability

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the table 4.

Inter day precision

Prepared and analyzed three replicate for day-1 and day-2 sample preparations as per method. And calculate the %RSD of assay level. The results were tabulated in Table 5.

Accuracy (Recovery)

Demonstrated the accuracy of the test method by preparing recovery samples (i. e. spiking formulation with known quantities of API.) at

the level of 50 %, 100 %, and 150 % of target concentration. Prepare the recovery sample in triplicate in each level. The sample was prepared as like assay method. The results were tabulated in Table 6,7,8.

Limit of detection and quantification (LOD and LOQ)

From the linearity data calculate the limit of detection and quantitation, using the following formula. The results were tabulated in Table 9.

$$LOD = \frac{3.3\,\sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyse

$$LOQ = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyse

Robustness

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but were still within the specified parameters of the assay by changing physical parameters like flow rate mobile phase and pH. HPLC system was set with small but deliberate change in method as mentioned below and their effect on system suitability test and the values were given in Table 10.

Assay

Triumeq (600+300+50) from Vie Healthcare, baring the label claim Lamivudine 600mg Abacavir 300mg Dolutegravir 50mg per unit formulation Assay was performed with the above formulation. The results were tabulated in Table 11, 12, 13.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. The results were tabulated in Table 14, 15, 16.

DISCUSSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 225nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area (figure 4). The column used on C18 150x4.6mm, 5µ. was chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 0.8ml/min because of good peak area and satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase with ratio of 65:35 buffers: Acetonitirile was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Diluent methanol was selected because of maximum extraction, sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 12min because analyze gave peak around 4.0 and also to reduce the total run time. The percent recovery was found to be 99.95% 99.96% and 99.66% was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. In specificity study all degradant impurity and excipient peaks were separated from the analyse peak. Detection limit was found to be 0.001µg/ml. Linearity study was, correlation coefficient and curve fitting was found to be 10µg/ml. The analytical method was found linearity over the range of 2-10µg/ml of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was all satisfactory

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Abacavir, Lamivudine and Dolutegravir in Tablet dosage form. Retention time of Abacavir, Lamivudine and Dolutegravir were found to be 2.338 min, 3.276 min and 3.870 min. %RSD of system precision for Abacavir, Lamivudine and Dolutegravir were and found to be 0.4, 0.4 and 0.4 respectively. %RSD of method precision for Abacavir, Lamivudine and Dolutegravir. were and found to be 0.8, 0.7 and 0.8 respectively. % recovery was obtained as 99.95%, 99.96% and 99.66% for Abacavir, Lamivudine and Dolutegravir Respectively. LOD values are obtained from regression equations of Abacavir, Lamivudine and Dolutegravir. Were 0.75ppm, 1.80ppm, 0.02ppm and LOQ values are obtained from regression equations of Abacavir, Lamivudine and Dolutegravir? Were 2.288ppm, 2.28ppm, 1.80ppm respectively Regression equation of Abacavir. Was y = 15367x + 28649, Lamivudine was y = 23710x + 24093 and of Dolutegravir was y = 12067x + 1398. Retention times are decreased so the method developed was simple and economical that

can be adopted in regular Quality control test in Industries.

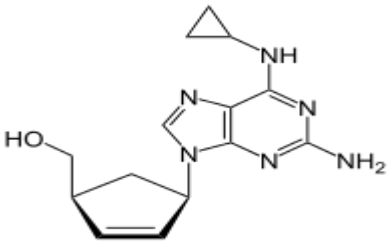

Fig. 1: Chemical Structure of Abacavir

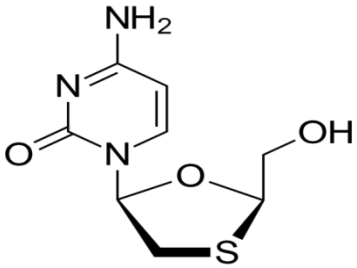

Fig. 2: chemical structure of Lamivudine

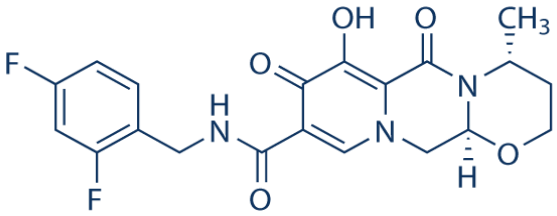

Fig. 3: Chemical Structure of Dolutegravir

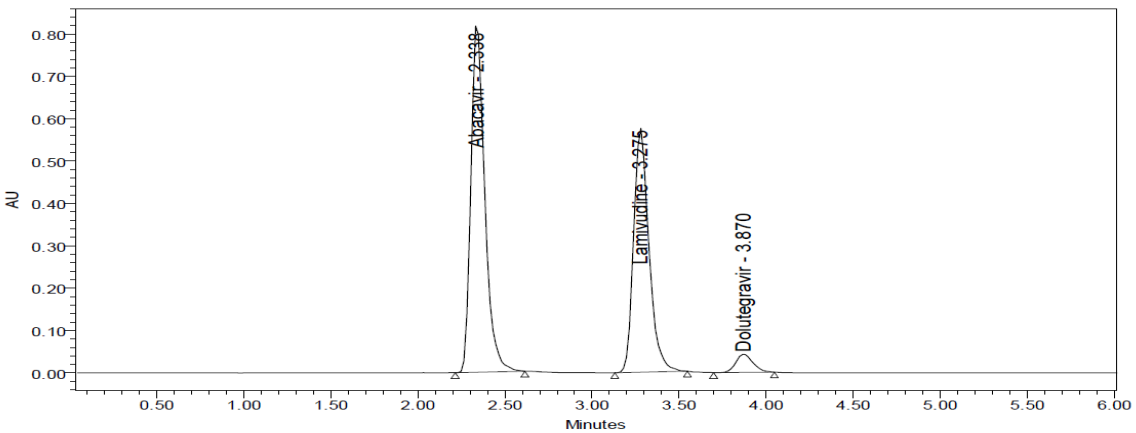

Fig. 4: RP-HPLC data of Abacavir Lamivudine and Dolutegravir

Table 1: System suitability parameters for Abacavir, Lamivudine, and Dolutegravir

S no	Abacavir			Lamivudine			Dolutegravir		
Inj	RT(min)	TP	Tailing	RT(min)	TP	Tailing	RT(min)	TP	Tailing
1	2.337	4563	1.38	3.273	6231	1.27	3.860	7218	1.18
2	2.338	4067	1.35	3.274	6221	1.27	3.862	8063	1.16
3	2.338	5098	1.33	3.276	6192	1.26	3.864	7463	1.18
4	2.338	4598	1.34	3.276	7187	1.21	3.865	6727	1.15
5	2.338	4340	1.28	3.277	7060	1.25	3.868	7039	1.14
6	2.339	4013	1.38	3.278	6571	1.27	3.870	7347	1.16

Table 2: Linearity for Abacavir, Lamivudine and Dolutegravir

Abacavir		Lamivudine		Dolutegravir	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
75	1251999	37.5	968599	6.25	77956
150	2304223	75	1779302	12.5	157911
225	3470543	112.5	2670001	18.75	224377
300	4623169	150	3578843	25	298578
375	5806354	187.5	4497785	31.25	377238
450	6948004	225	5345952	37.5	457476

Table 3: System precision table of Abacavir, Lamivudine and Dolutegravir

S. No	Area of Abacavir	Area of Lamivudine	Area of Dolutegravir
1.	4686224	3612387	302289
2.	4636238	3593729	300961
3.	4672357	3614257	300068
4.	4652001	3618927	300974
5.	4658919	3627707	300120
6.	4690287	3629255	303384
Mean	4661960	3616044	301299
S.D	20838.3	12910.4	1300.2
%RSD	0.4	0.4	0.4

Table 4: Repeatability table of Abacavir, Lamivudine and Dolutegravir

S. No	Area of Abacavir	Area of Lamivudine	Area of Dolutegravir
1.	4625380	3601311	299816
2.	4659369	3627867	298874
3.	4609685	3673450	299896
4.	4676038	3654222	304482
5.	4597715	3642452	302870
6.	4679396	3626342	298963
Mean	4641264	3637607	300817
S.D	35034.6	24993.7	2311.3
%RSD	0.8	0.7	0.8

Table 5: Intermediate precision table of Abacavir, Lamivudine and Dolutegravir

S. No	Area of Abacavir	Area of Lamivudine	Area of Dolutegravir
1.	4564106	3529797	291524
2.	4552453	3555081	299693
3.	4506063	3532325	294205
4.	4595735	3594791	297008
5.	4551771	3560497	291616
6.	4572979	3563144	297291
Mean	4557185	3555939	295223
S.D	29828.2	23765.2	3321.8
%RSD	0.7	0.7	1.1

Table 6: Accuracy table of Abacavir

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	%Recovery	Mean %Recovery
50%	150	149.04	99.36	99.95
	150	151.69	101.13	
	150	148.92	99.28	
100%	300	297.49	99.16	
	300	300.09	100.03	
	300	300.91	100.30	
150%	450	447.09	99.35	
	450	451.92	100.43	
	450	452.17	100.48	

Table 7: Accuracy table of Lamivudine

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	%Recovery	Mean %Recovery
50%	75	74.76	99.68	99.96
	75	75.97	101.29	
	75	75.07	100.09	
100%	150	150.32	100.21	
	150	149.41	99.61	
	150	147.14	98.09	
150%	225	226.52	100.67	
	225	225.38	100.17	
	225	224.53	99.79	

Table 8: Accuracy table of Dolutegravir

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	%Recovery	Mean %Recovery
50%	12.5	12.54	100.31	99.66
	12.5	12.58	100.62	
	12.5	12.49	99.93	
100%	25	24.71	98.85	
	25	24.80	99.21	
	25	25.06	100.22	
150%	37.5	37.03	98.75	
	37.5	37.26	99.37	
	37.5	37.37	99.67	

Table 9: Sensitivity table of Abacavir, Lamivudine and Dolutegravir

Molecule	LOD(µg/ml)	LOQ(µg/ml)
Abacavir	0.75	2.28
Lamivudine	1.80	5.47
Dolutegravir	0.02	0.07

Table 10: Robustness data for Abacavir, Lamivudine and Dolutegravir

S.no	Condition	%RSD of Abacavir	%RSD of Cilnidipine	%RSD of Dolutegravir
1	Flow rate (-) 0.7ml/min	0.6	0.8	0.7
2	Flow rate (+) 0.9ml/min	0.4	0.4	1.2
3	Mobile phase (-) 70B:30A	1.0	0.2	1.1
4	Mobile phase (+) 60B:40A	0.5	0.7	1.0
5	Temperature (-) 25°C	0.1	0.7	1.2

6	Temperature (+) 35°C	0.5	0.7	0.8

Table 11: Assay Data of Abacavir

S.no	Standard Area	Sample area	% Assay
1	4686224	4625380	99.02
2	4636238	4659369	99.74
3	4672357	4609685	98.68
4	4652001	4676038	100.10
5	4658919	4597715	98.42
6	4690287	4679396	100.17
Avg	4661960	4641264	99.36
Stdev	20838.3	35034.6	0.750
%RSD	0.4	0.8	0.8

Table 12: Assay Data of Lamivudine

S.no	Standard Area	Sample area	% Assay
1	627909	621933	99.23
2	624112	626052	99.88
3	628450	624919	99.70
4	626366	622416	99.30
5	625325	620675	99.02
6	621050	623247	99.43
Avg	625535	623207	99.43
Stdev	2720.6	1984.3	0.32
%RSD	0.4	0.3	0.32

Table 13: Assay Data of Dolutegravir

S.no	Standard Area	Sample area	% Assay
1	302289	299816	99.41
2	300961	298874	99.10
3	300068	299896	99.43
4	300974	304482	100.96
5	300120	302870	100.42
6	303384	298963	99.13
Avg	301299	300817	99.74
Stdev	1300.2	2311.3	0.766
%RSD	0.4	0.8	0.8

Table 14: Degradation Data of Abacavir

S.No	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.65	0.202	0.280
2	Alkali	3.00	0.163	0.489
3	Oxidation	1.87	0.250	0.280
4	Thermal	0.68	0.145	0.286
5	UV	0.68	0.100	0.286
6	Water	0.79	0.143	0.285

Table 15: Degradation Data of Lamivudine

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.56	0.115	0.428
2	Alkali	2.88	0.128	0.489
3	Oxidation	1.79	0.116	0.411
4	Thermal	0.96	0.129	0.455
5	UV	0.70	0.125	0.484
6	Water	0.117	0.117	0.448

Table 16: Degradation Data of Dolutegravir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.97	0.342	0.365
2	Alkali	2.90	0.252	0.376
3	Oxidation	1.97	0.209	0.974
4	Thermal	0.93	0.284	0.371
5	UV	0.94	0.333	0.381

6	Water	0.89	0.332	0.386

ACKNOWLEDGEMENTS

The author of thankful to authorities of department of pharmaceutical analysis ,vikas college of pharmacy, jntuk university, Vissannapeta for providing necessary facilities, authors are also thankful to authorities of departments, the authors are thankful to spectrum labs.

REFERENCES

1. Chatwal and Anand KS. High performance liquid chromatography, Instrumental methods of chemical analysis. Himalaya publishers: Mumbai. 2010;5th edition 2.570-2.629.
2. Sharma. High performance liquid chromatography. Instrumental methods of chemical analysis, Goel publishers, Meerut. 2005; 24th edition 295-300.
3. Dong HPLC Instrumentation and trends. Modern HPLC for practicing scientists. USA. 2006; 5th edition 78-110.
4. Skoog H, DM West and Holler FJ. Fundamentals of Analytical Chemistry, Saunders College Publishing, Philadelphia.1992;9th edition 1-3.
5. Corners KA. Textbook of Pharmaceutical Analysis, A Wiley- inter science Publication.1967;1st edition 475-478.
6. Kasture KA, Wadodkar SG, Mahadik KR and More HN. Textbook of Pharmaceutical Analysis – II, Published by Nirali Prakashan. 2005;25th edition 256-260.
7. Beckett and Stanlake AH. J.B. Practical Pharmaceutical Chemistry, Part 2, CBS Publishers and Distributors. 2002; 4thedition 157-174.
8. Snyder, Kirklannd JJ and Glaijch LJ. Practical HPLC method development, New York. 1997;2ed edition 30-100.
9. Satinder and Dong MW. Method development and validation. Pharmaceutical analysis by HPLC. Newyork. 2005; 15th edition 16-70.
10. Swartz and Ira Krull. Analytical method development and validation, Marcel Dekker, New York. 2009;1st edition 17-80.
11. Rajkumar Prava, Ganapathy Seru, Vamsi Krishna Pujala and Surendra Babu Lagu. RP-HPLC method development and validation for the simultaneous determination of lamivudine, abacavir and dolutegravir in pharmaceutical dosage forms. World J Pharm Sci. 2017;35(5):565- 365.
12. Saidulu P and Mastanamma SK. Stability Indicating Gradient RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Abacavir and Dolutegravir in bulk and their Combined Dosage Form. Int J Pharm Sci. 2016;37(2):249- 257.
13. Valli Purnima B, Santha Kumari M, Ramu G and Vijaya Bhaskara Reddy T. Validated Reversed Phase HPLC Method for Assay and Degradation Studies of Lamivudine, Abacavir Sulphate And Dolutegravir In Combined Dosage Form. Int J Res. 2016;6(20):1560-1565.
14. Pal N, Srinivas A and Ravikumar P. Simultaneous HPLC Method Development and Validation for Estimation of Lamivudine, Abacavir and Dolutegravir in Combined Dosage Form with their Stability Studies. Asian journal of chemistry. 2016;2(28):273-276.
15. Nagubandi N and Nagaraju P. Simultaneous estimation of abacavir, lamivudine and dolutegravir from its tablet dosage form by UPLC method. World J Pharm Sci. 2015;3(10):2135-2140.