WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 4, 1506-1512. Research Article ISSN 2277 – 7105

DEVELOPMENT OF VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ABACAVIR, LAMIVUDINE AND ZIDOVUDINE IN COMBINED TABLET DOSAGE FORM

Vaishali P. Nagulwar[1]*, Kishor P. Bhusari[2]

[1].Government College of Pharmacy, Kathora Naka, Amravati-444604, Maharashtra, India.

[2].Sharad Pawar College of Pharmacy, Wanadongri, Nagpur-441110, Maharashtra, India.

Article Received on
30 April 2014,
Revised on 25 May 2014,
Accepted on 16 Jun 2014

***Correspondence for Author**
Vaishali P. Nagulwar
Government College of Pharmacy, Kathora Naka, Amravati-444604, Maharashtra, India.

ABSTRACT

Purpose: To develop an accurate, simple and reproducible RP-HPLC method for the simultaneous estimation of abacavir, lamivudine and zidovudine in bulk drug and in combined tablet dosage form. **Method:** The stock solutions were prepared in acetonitrile and methanol (3:2) followed by the further required dilutions with distilled water. The mobile phase containing methanol: buffer (pH 3.0): acetonitrile: tetrahydrofuran (35:60:5:0.4 v/v/v/v) was used at 271nm at a flow rate of 0.6 mL/min. HPLC model Chemito LC 6600 equipped with Knauer HPLC pump K-501 and Chemito UV-visible detector connected to Chemito's Chemitochrome C2000 data module, Eurosphere 100-5 C_{18} (250 mm x 4.6 mm) with precolumn were used in present study. Validation parameters like accuracy, precision, linearity, specificity and ruggedness were performed to ascertain the correctness of the proposed method. **Result:** The proposed method has estimated abacavir 99.12% ± 0.64, lamivudine 99.55% ± 0.6 and zidovudine 99.76% ± 0.4 in standard mixture and 99.26% ± 0.49, 99.67% ± 0.51 and 99.68% ± 0.58 respectively in the marketed tablets. **Conclusion:** The present study gives an excellent method for the determination of all the three drugs in combined dosage formulation.

KEYWORDS: ABA, LAM, ZID, RP- HPLC.

INTRODUCTION

Abacavir (ABA), Lamivudine (LAM) and Zidovudine (ZID) belongs to potent nucleoside analog reverse transcriptase inhibitor class of antiretroviral drugs. Literature review reveals

some HPLC, [1]-[4] LC-MS/MS [5] and UV spectrophotometric [6] methods for their estimation in pharmaceuticals and biological samples. None of the method has been found to estimate the drugs in said combination as per the proposed technique. Hence the present method was developed to quantify ABA, LAM and ZID in tablet dosage form by RP-HPLC.

MATERIALS AND METHODS

Instrumentation and Materials

HPLC model CHEMITO LC 6600 equipped with KNAUER HPLC pump K-501 and CHEMITO UV-visible detector connected to CHEMITO'S CHEMITOCHROME C2000 DATA MODULE, Eurosphere 100-5 C_{18} (250 mm x 4.6 mm) with precolumn, pH meter model Environmental-Scientific Inst.Co-1012-E, Shimadzu's 1601 UV–visible spectrophotometer with a matched pair of 10mm quartz cells were utilized in the present study. Chemicals like Acetonitrile, methanol, tetrahydrofuran, potassium dihydrogen phosphate and o-phosphoric acid (LOBA, India Ltd), distilled water, pure drugs of ABA, LAM and ZID (Cipla Ltd. Patalganga and Kurkumbh, INDIA) and tablets containing a combination of ABA-300mg, LAM-150mg and ZID-300 mg were used in the development of the proposed method.

Method

Preparation of Standard Stock Solutions*:* An accurately weighed quantity of ABA, LAM and ZID (50 mg each) were transferred to three separate volumetric flasks (50 mL) and were dissolved in acetonitrile (30 mL) a nd the volume was made up to 50 mL with methanol to get solutions having concentration 1000 µg/mL. Following chromatographic conditions were fixed and maintained throughout the method.

Column : Eurosphere 100-5 C_{18} (250 x 4.6 mm) with precolumn
Detection wavelength : 271 nm
Flow rate : 0.6 mL/min
Temp : Ambient
Injection volume : 20 µL
Mobile phase : Methanol: buffer (pH 3.0):acetonitrile:tetrahydrofuran (35:60:5:0.4 v/v/v/v)

The chromatographic conditions were set as per the given parameters and mobile phase was allowed to equilibrate with stationary phase. Standard drug solution (ABA, LAM and ZID) were injected in the mobile phase separately. The chromatogram is shown in Fig.1.

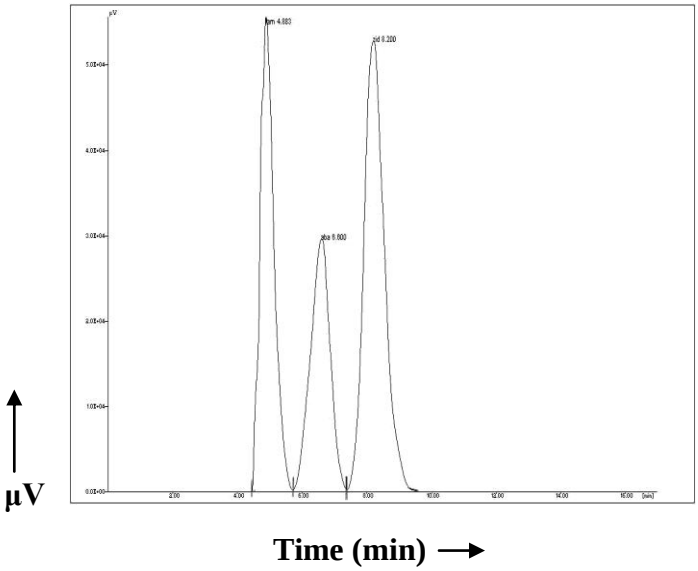

Fig.1: Chromatogram of ABA, LAM and ZID

Study of linearity range

The standard drug solutions were diluted with distilled water to get different concentrations ranging from LAM as 5-15 µg/mL, ABA and ZID as 10-30 µg/mL respectively. The mobile phase was allowed to equilibrate with stationary phase. Each of the standard solution was injected separately and the chromatograms were recorded. The linearity graph was plotted as concentration against detector response (peak area). The correlation coefficient (r) for ABA, LAM and ZID were found to be 0.985, 0992 and 0.988 respectively.

Study of system suitability parameter

The system suitability tests were carried out and the results of system suitability tests are shown in Table 1.

Table 1: Data of system suitability tests

Drug	Retention time(min)	Resolution	Capacity factor	Column efficiency Theoretical plates	Asymmetric factor
				Per column (30cm)	
ABA	6.61	1.922	1	595.31	0.91
LAM	4.87	0.000	1	709.89	1.32
ZID	8.21	1.435	1	816.93	1.21

Analysis of laboratory mixture by proposed method

Standard stock solutions of ABA, LAM and ZID were mixed in the ratio 2:1:2 respectively. The aliquot portions of stock solution of ABA, LAM and ZID were diluted with distilled water to get concentration of 10:5:10, 15:7.5:15, 20:10:20, 25:12.5:25 and 30:15:30 µg/mL of

ABA, LAM and ZID, respectively. The chromatographic conditions were set as detailed under the study of linearity range (10:5:10, 15:7.5:15, 20:10:20, 25:12.5:25 and 30:15:30 µg/mL) and each standard laboratory mixture was injected separately. The chromatograms were recorded and the concentration of each drug was estimated by comparing peak area with standard. The results of estimation of standard laboratory mixture are shown in Table 2.

Table 2: Analysis of standard laboratory mixture

S. No.	Drug taken(µg/mL)			% of drug estimated		
	ABA	LAM	ZID	ABA	LAM	ZID
1	10	5	10	99.52	98.69	100.19
2	15	7.5	15	99.71	99.77	100.05
3	20	10	20	99.45	100.02	99.65
4	25	12.5	25	98.23	100.10	99.77
5	30	15	30	98.67	99.17	99.14

Drug	Mean	±SD	SE	CV
ABA	99.12	0.64	0.28	0.0064
LAM	99.55	0.60	0.27	0.0060
ZID	99.76	0.40	0.18	0.0040

Analysis of tablets by proposed method

Tablets containing ABA (300 mg), LAM (150 mg) and ZID (300 mg) were finely powdered. An accurately weighed quantity of tablet powder equivalent to about ABA (25 mg) was dissolved in acetonitrile (15 mL) in volumetric flask (25 mL). The volume was made up to 25 mL with methanol. The solution was filtered. The filtrate was diluted with distilled water to get the concentration of ABA equivalent to 20 µg/mL. The equal volume of standard and sample solution was injected separately and the chromatograms were recorded. The contents of ABA, LAM and ZID were calculated and the results are shown in Table 3.

Table 3: Assay of ABA, LAM and ZID tablets by proposed method

S. No.	Quantity of tablet powder (g)	Amount of drug estimated(g/tab)			% of label claimed		
		ABA	LAM	ZID	ABA	LAM	ZID
1	0.1877	0.2975	0.1503	0.2960	99.15	100.15	98.69
2	0.1876	0.2963	0.15.2	0.2991	98.74	100.09	99.70
3	0.1875	0.2966	0.1496	0.2995	98.85	99.69	99.82
4	0.1877	0.2993	0.1493	0.3003	99.76	99.51	100.08
5	0.1877	0.2994	0.1484	0.3004	99.78	98.89	100.12

Drug	Mean	±SD	SE	CV
ABA	99.26	0.49	0.22	0.0049
LAM	99.67	0.51	0.23	0.0051
ZID	99.68	0.58	0.26	0.0058

Average weight of tablet = 1.12615 g (Each tablet stated to contain ABA- 300 mg, LAM 150 mg and ZID- 300 mg).

Recovery studies

To an accurately weighed quantity of preanalysed tablet powder equivalent to 25 mg of ABA in volumetric flask (25 mL), about (5 mg each) of pure ABA, LAM and ZID were added. The content was shaken for 15 min with acetonitrile (15 mL) and then the volume was made up to 25 mL with methanol. The solution was then filtered. An aliquot portion of the resultant solution was appropriately diluted with distilled water to get final concentration within the range of mixed standard. The results of recovery studies for ABA, LAM and ZID were found to be satisfactory as 99.47 ± 0.48, 99.1 1± 0.36 and 99.3 ± 0.45 respectively.

Validation parameters

Validation of the proposed method was carried out as per ICH guidelines. The results of the studies of validation parameters like accuracy, precision, specificity and ruggedness are given in Table 5.

Table 5: Results of validation studies

Parameter	**% of label claim**		
	ABA	**LAM**	**ZID**
Accuracy	99.47	99.11	99.3
Precision	99.26	99.67	99.68
(Specificity) Normal	95.85	97.56	97.74
Alkali	23.2	45.4	48.7
Acid	28.4	46.4	49.04
Oxide	32.03	43.2	48.7
Ruggedness: Different analyst and different days			
1	99.50, 99.76	99.63, 99.51	98.85, 99.84
2	98.72, 99.82	98.74, 99.47	99.07, 98.91
3	99.81, 98.76	99.85, 99.57	99.43, 99.73

RESULTS AND DISCUSSION

The proposed RP-HPLC method includes simultaneous determination of ABA, LAM and ZID in pharmaceutical formulation. The drugs shows better resolution in methanol: acetonitrile: buffer pH 3.0: tetrahydrofuran (35:5:60:0.4 v/v/v/v) as compared to different proportions of mobile phases. A flow rate was 0.6 mL/min. and the analysis was performed using UV detector at wavelengths 271 nm. The retention times of ABA (6.61min), LAM (4.87 min) and ZID (8.21 min). The peaks were having good resolution with asymmetric factor 0.91 for

ABA, 1.32 for LAM and 1.21 for ZID. The calibration curves showed linearity over the concentration range from 10-30 µg/mL for ABA and ZID and 5-15 µg/mL for LAM. The r values obtained with linear regression curve were above 0.98. Linearity data shows that intensity of detector response is proportional to the concentration of analyzed substance. The SD amongst replicate responses was less than 1% indicating precision of the method. The mean recoveries obtained for ABA, LAM and ZID were 99.47, 99.11 and 99.3, respectively, which confirms accuracy of the proposed method

CONCLUSION

The main advantage of the proposed method is its suitability for routine determination of ABA, LAM and ZID from their marketed tablet formulations since the proposed method is simple, economical, precise and reproducible. Therefore it can be adopted for the estimation of the drugs in said fixed dose combination.

ACKNOWLEDGMENTS

The authors are thankful to Cipla Ltd. Kurkumbh and Patalganga, India, for giving gift samples of pure drugs. They are also grateful to the Director, Sharad Pawar College of Pharmacy, Nagpur for providing necessary facilities for research work.

REFERENCES

1. Djurdjevic P, Laban A, Markovic S, Stankov MJ. Chemometrically optimized RP-HPLC method for the estimation of Abacavir, Lamivudine and Zidovudine in tablets. *Anal Lett,* 2004; 37: 2649-67.
2. Kumar DA, Rao GS, Rao S. Simultaneous method for the determination of Lamivudine, Zidovudine and Abacavir in tablet dosage forms by RP-HPLC. *E-J. Chem,* 2010; 7:180-84.
3. Raja T, Lakshmana Rao A. Validated RP-HPLC method for the estimation of Abacavir, Lamivudine and Zidovudine in pharmaceutical dosage form. *Int. J. Pharm. Tech. Res,* 2011; 3: 852- 57.
4. Pai NR, Desai AS. Simultaneous estimation method for some retroviral drugs from tablets by RP- HPLC method. *Ind. Drugs,* 2005; 42: 681-84.
5. Robbins B, Poston PA, Neal EF, Slaughter C, Rodman JH. Simultaneous method for measurement of intracellular triphosphate metabolites of Zidovudine, Lamivudine and Abacavir (carbovir) in human peripheral blood mononuclear cells by combined anion exchange solid phase extraction and LC–MS/MS. *J. Chromatogr. B,* 2007; 850: 310-17.

6. Nagulwar VP, Bhusari KP. Simultaneous estimation of Abacavir, Lamivudine and Zidovudine in combined tablet dosage form by UV spectrophotometric method. *Int. J. Res. Ayurved. Pharm*, 2011; 2: 610-14.