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Simultaneous U.V. Spectrophotometric estimation of Lamivudine and Abacavir Sulphate in bulk and in tablet dosage form

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ABSTRACT

A Simultaneous ultraviolet spectrophotometric method has been developed for the analysis of LAM and ABA in the combined dosage form (Abamune-L). The method depends on the application of simultaneous equation to resolve the interference due to spectral overlapping. The analytical signals were measured at 270 and 289 nm using 0.1 N HCl as a solvent. Regression analysis of Beer's plot showed good correlation in a general concentration range of 5 to 30 µgml-1 with correlation coefficient (r= 0.9995; CV < 0.7022) for lamivudine, whereas abacavir concentration range 5 to 30µg/ml with correlation coefficient (r= 0.9992; CV <0.5151). These methods were validated with respect to accuracy, precision, linearity, limit of detection and quantification. The suggested procedures were successfully applied to the determination of these compounds in pharmaceutical preparations, with high percentage of recovery, good accuracy and precision.The results of analysis have been validated statistically by repeatability and recovery studies. The results were found satisfactory and reproducible. The method was applied successfully for the estimation of LAM and ABA simultaneously in tablet form with out the interference of excipients

Key words: Lamivudine, Abacavir sulphate, Simultaneous estimation, Validation, UV Spectrophotometric method.

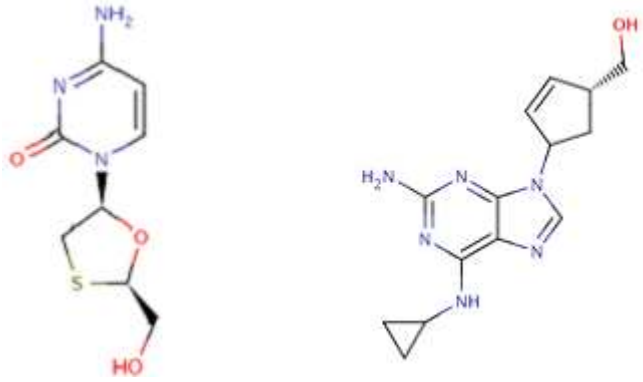
INTRODUCTION

The combination of Lamivudine (LAM) and Abacavir (ABA) has recently been introduced in the market. Chemically Lamivudine [1] is (Lam) 4 amino-1- [2R, 5S)-2-(hydroxyl methyl)-1, 3-oxathiolan-5yl] pyrimidine-2-one. Lamivudine is a nucleoside reverse transcriptase

inhibitor (NRTI) with an activity against human immunodeficiency virus type (1) (HIV)1 and hepatitis B. Abacavir[2] is (Aba) [1R]-4-(2 amino-6-cyclopyridine) purine-9-yl) -1-cyclopent-2enyl] methanol. It is a nucleoside reverse transcriptase inhibitor (NRTI) with an activity against human immune deficiency virus Type (1) (HIV1).

The drugs are prescribed individually, as well as multicomponent dosage forms available in the market. A number of methods have been published for the estimation of above said analytes.

Lamivudine **Abacavir Sulphate**



Spectrophotometric estimation of Abacavir sulphate [3]. Spectrophotometric estimation of Lamivudine [4], Lamivudine in human plasma by RP-HPLC [5]. Titrimetric and spectrophotometric estimation of Lamivudine[6]. Methods were reported for simultaneous analysis of Abacavir and Lamivudine in human plasma by LC/MS/MS[7]. Determination of Abacavir, Lamivudine & Zidovudine in pharmaceutical tablets human serum and in drug dissolution studies by HPLC [8] was also reported in the literature.

Literature survey reveals that so, far no simultaneous uv method has been reported for the simultaneous estimation of Lam and Aba formulation. In the present investigation, an attempt have been made to develop a rapid, accurate, precise and cost effective uv method for simultaneous uv estimation of Lam and Aba in combined dosage form.

EXPERIMENTAL SECTION

Lamivudine and Abacavir pure drug sample were generously gifted by Dr. Reddy's Laboratories, Hyderabad. The formulation Abamune-L was procured from the local pharmacy. A Systronics double beam UV-VIS spectrophotometer with 1cm matched quartz cells were used for the all absorbance measurements. All the chemicals and reagents used were of analytical grade and procured from Qualigens India Ltd, India.

Solubility Studies

The solubility of the Lamivudine and Abacavir were studies as per I.P using many polar and non-polar solvents. It was found that both the drugs were freely soluble in 0.1N HCL was selected for as the solvent of choice further studies.

Standard Stock Solution

Lamivudine and Abacavir were weighed separately (100mg) and dissolved in 0.1N HCL and made up to 100 ml in volumetric flasks to get a concentration of 1000µg/m**l.**

Selection of λ_{Max}

The standard stock solutions of Lamivudine and Abacavir further diluted separately to get a concentration of 10µg/ml. The absorbance of the solutions were scanned in the UV region and found that Lam showed maximum absorbance at 270 and Aba at 289nm. Thus λmax of Lam was found to be 270 nm and Aba was found to be 289 nm.

Figure-1: UV Spectrum of Lamivudine in 0.1N HCL

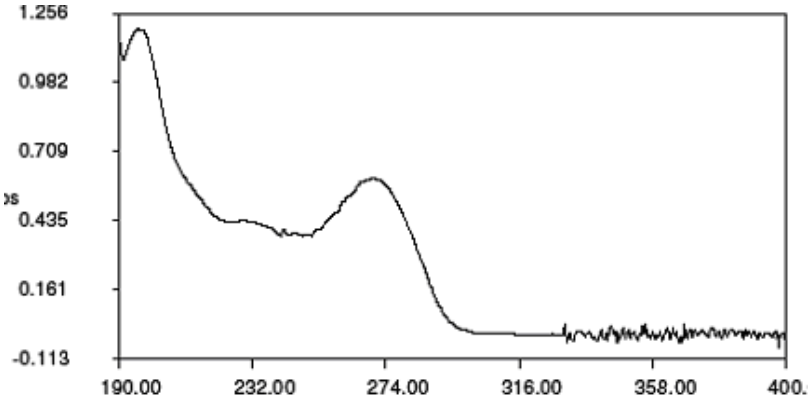


Figure-2: UV Spectrum of Abacavir in 0.1N HCL

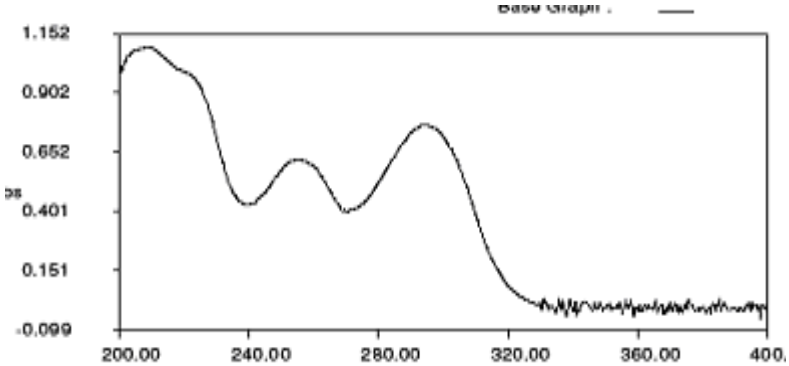
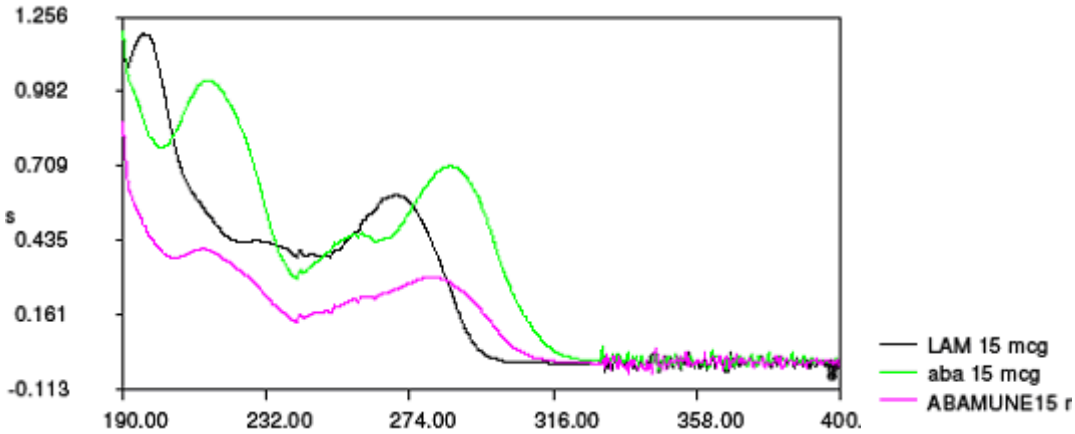


FIGURE-3: UV-Spectrum of Abamune-L in 0.1N HCL (Zero Overlap Spectrum)



Stability Studies

The standard stock solution of Lamivudine was diluted to get a concentration 10µg/ml and stability studies were performed. It was found that Lam was stable for 1 hour and 45 minutes. Similarly studies were performed on Aba and found that Aba was stable for 1 hour and 30 minutes.

Calibration Graph

The standard stock solution of Lamivudine was diluted to get a concentration ranging 5-30µg/ml.The absorbance's of resulting solutions were measured at 270 nm. Similar procedure was followed for Abacavir and absorbance measured at 287 nm. It was found that the Lam and Aba showed good linearity at concentrations ranging 5-30µg/ml.

Quantification of Raw Materials

Standard stock solution of Lamivudine was diluted to obtain a concentration of 10µg/ml and the absorbance measured at 270 nm. The procedure was repeated for six times. Similar studies were performed on Aba. The amount of Lam and Aba present respectively were calculated from the slope and intercept on the respective calibration graph.

Quantification of Formulation by Simultaneous Method [9]

ABAMUNE-L tablets were used for the study. Ten tablets were used and tablet powder to 250 mg of Abacavir was dissolved in 0.1HCL, sonicated, filtered and made up to 100 ml using 0.1N HCl. A concentration of 15 µg/ml was prepared using this solution the absorbance of the solution was measured simultaneously at 270 and 289nm. The amount of Lam and Aba was determined using simultaneous equation. The procedure was repeated for six times.

Recovery Studies

The Accuracy of the method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (20%, 40%, 60%) with in the range of linearity for both the drugs. The result of analysis of recovery studies obtained by the method was validated by statistical evaluation**.**

Repeatability

Repeatability was given by interday and intraday precision. The assay and recovery procedure was repeated for three times on the same day and one time on the successive days.

RESULTS AND DISCUSSION

The drugs Lam and Aba were soluble in 0.1N HCL. λmax of Lam was found to be 270nm and that of Aba was 287 nm at 15µg/mL. The spectrums of Lam & Aba were shown in fig-1&2. Concentrations ranging 5-30µg/mL of Lam and Aba raw materials were studied separately and it was found that the drugs showed good linearity at the concentration range of 5-30 µg/mL. The Correlation coefficient r^2 value (n=3) were found to be 0.9995 and 0.9992 respectively. The results were shown in table-1. Quantification of raw materials that15 µg/mL of Lam and 15 µg/mL of Aba were performed and the percentage purity was found to be 99.68% to100.30%. The results were shown in table 2&3. The tablet formulation of Lam and Aba- Abamune-L was quantified using tablet powder containing equivalent to 100mg of lam in 0.1N HCL. Both the drugs were analysed simultaneously at the two λmax 270nm and 289nm. The amount of Lam and Aba were calculated by using simultaneous equation. The proposed method was validated as per ICH guidelines. [10, 11] The percentage purity of Lam and Aba in the tablet formulation was found to be 100.01to 99.50%. The percentage RSD [12] values at the two drugs were calculated to 0.8819 and 0.7854 respectively. The low percentage RSD values indicate that the developed method has good precision. The results were shown in Table-4. The zero overlap spectrums were shown in fig-3. The precision of the method was further confirmed by interday and intraday analysis. The percentage purity value of the intraday and interday analysis of Abamune-L was found to be 100.22 and 99.45 for Lamivudine and 100.65 and 99.45 for Abacavir. The results were shown in Table-5.The accuracy of the method was performed by recovery studies.

Table-1: Optical Characteristics of Lamivudine & Abacavir by Simultaneous UV-Method

Parameters	Lamivudine	Abacavir
λ-max (nm)	270	289
Beer s law limit	5-30µg/ml	5-30 µg/ml
Sandell's sensitivity	0.02331	0.03317
Molar absorptivity	13915.47	12025.86
Correlation coefficient	0.9995	0.9992
Regression equation	Y=0.0430+0.002375	Y=0.0301+0.00014
Slope(m)	0.0430	0.0301
intercept	0.002375	0.00014
LOD	0.6295	0.0383
LOQ	1.9077	0.1160

Table-2: Quantification of Raw Material-Lamivudine

S. No	Expected Amount (µg/ml)*	Amount Found (µg/ml)*	Percentage Purity (%)	Average (%)	S.D	%RSD
1.	15.00	15.11	100.73			
2.	15.00	14.90	99.33			
3.	15.00	14.85	99.00	99.68	0.7000	0.7022
4.	15.00	14.90	99.33			
5.	15.00	15.06	100.40			
6.	15.00	14.90	99.33			

**Mean of six observations*

Table-3: Quantification of Raw Material-Abacavir

S. No	Expected Amount (µg/mL)*	Amount Found (µg/mL)*	Percentage Purity	Average (%)	S.D	%RSD
1.	15.00	14.92	99.66			
2.	15.00	15.01	100.07			
3.	15.00	15.10	100.66	100.30	0.5167	0.5151
4.	15.00	15.04	100.26			
5.	15.00	15.17	100.13			
6.	15.00	15.01	100.07			

**Mean of six observations*

Table-4: Quantification of Formulation Abamune-L

Formulation	Drugs	Label Claim	Amount found	% purity	Average	SD	% RSD
Abamune-L	Lamivudine	300mg	298.642	99.54	100.01	0.4300	0.4299
			301.604	100.53			
			300.114	100.03			
			299.190	99.73			
			299.164	99.72			
			301.604	100.53			
	Abacavir	600mg	599.190	99.86	99.50	0.5911	0.5940
			603.285	100.54			
			594.190	99.03			
			595.266	99.21			
			596.190	99.36			
			594.285	99.04			

To the pre analysed formulation a known quantity of Lam and Aba raw material solution were added at different levels, 20%, 40%, 60%. The percentage recovery was found to be in the range of 99.11to 99.90%. The percentage RSD values were found to be less than 2%. The

reports of analysis were shown in Table-6. Further the precision of the method was confirmed by interday and intraday recovery studies. The percentage RSD values for Lam were found to be 0.1891-0.5098 and 0.2847-0.8817 for and intraday recovery analysis of formulation. The % RSD values for Aba were found to be 0.3591-0.5402 and 0.7625-01.0169 for interday and intraday recovery analysis of formulation. The results were shown in Table-7.

Table -5: Intraday and Interday Analysis of Formulation- Abamune –L

S. No.	Drugs (label claim)	Percentage obtained		S.D		% RSD	
		intraday	interday	Intraday	Interday	Intraday	Interday
1	lamivudine 300mg Mean	99.46 99.91 101.13 100.22	99.04 100.03 99.21 99.42	0.8665	0.5294	0.8646	0..5325
2	Abacavir 600mg Mean	100.66 100.70 100.51 100.65	99.86 99.36 100.54 99.92	0.1352	0.5922	0.1343	0.5926

Table-6: Recovery studies for formulation Abamune-L

Label claim	Amount Added	Amount Recovered	% Recovery	Average	SD	%RSD
Lamivudine 300mg	3.00 6.00 9.00	3.01 6.01 8.99	100.33 100.16 99.88	100.12	0.2272	0.2269
Abacavir 600mg	6.0 12.0 18.0	5.99 12.05 17.99	99.83 100.41 99.94	100.06	0.3080	0.3078

Table-7: Recovery studies for formulation (Abamune-L)

Interday and Intraday

Drug	Avg interday	Avg intraday	% RSD interday	%RSD intraday
Lamivudine	99.66 99.18 99.22	99.72 99.61 100.44	0.8817 0.2847 0.4158	0.5098 0.1891 0.3880
abacavir	98.99 99.72 100.18	100.10 99.91 100.03	1.0169 0.8642 0.7627	0.5402 0.5101 0.3591

CONCLUSION

Finally the present studies concluded that the developed UV simultaneous method for Lamivudine and Abacavir was easy, simple & accurate and thus it can be adopted for the estimation of drugs in combined dosage form and can be applied for routine analysis.

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