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KEY WORDS:

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF ABACAVIR SULFATE AND LAMIVUDINE IN TABLET DOSAGE FORM

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Two simple, accurate and economic methods Area under curve and Multicomponent have been described for the simultaneous estimation of Abacavir and Lamivudine in tablet dosage form. Abacavir shows absorption maximum at 284.0nm and Lamivudine shows absorption maximum at 270.0nm in distilled water. Beers law was obeyed in the concentration range of 5-30 µg/ml for Both Abacavir and Lamivudine. The coefficient correlations were found to be 0.9995 for LAM and 0.9992 for ABAC respectively.The method allows rapid analysis of binary pharmaceutical formulation with accuracy. Results of Tablet analysis by Multicomponent method was found to be 99.99% for ABAC and 100.03% for LAM while by AUC method it was found to be 99.99% for ABAC and 99.97% for LAM respectively. Results of analysis of two methods were validated statistically and by recovery studies and were found satisfactory.

Abacavir sulfate is {(1S, 4R)-4-[2-Amino-6-cyclopropyl amino) 9H-Purin-9yl) cyclopent-2-enyl) methanol sulfate. it works by preventing HIV from infecting new cells and taking them over. Abacavir is converted by cellular enzymes to the active metabolite, carbovir triphosphate (CBV-TP), CBV-TP inhibits the activity of HIV-1 reverse transcriptase (RT) by its incorporation into viral DNA.

Lamivudine Chemically it is (2R, 5S)-4-Amino1 [2-(Hydroxy methyl)-1, 3-oxathiolan-5yl]-2(1H)-Pyrimidinedione. It is used in HIV infection. Lamivudine is phosphorylated to its active 5'-triphosphate metabolite, lamivudine triphosphate (3TC-TP). The principal mode of action of 3TC-TP inhibition of RT via DNA chain termination after incorporation of the nucleotide analogue. Both Abacavir sulfate (ABAC) and Lamivudine (LAM) are official in IP[1]. Both the Drugs are marketed as combined dose tablet formulation and the Ratio is 300:600 mg LAM: ABAC. Literature survey revealed that a number of methods have been reported for estimation of Abacavir sulfate individually or in combination with other drugs and Lamivudine or in combination with other drugs[2-13]. Present work describes two simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of ABAC and LAM in tablet formulation.

A Jasco UV-VIS spectrophotometer model UV V-630 spectrophotometer was employed (spectral bandwidth 2 nm) with a pair of 1 m quartz cell for the method of AUC and UV 1800 shimadzu (UV /Vis spectrophotometer) spectral bandwidth 2 nm with a pair of 1 m quartz cell employed for the method of Multicomponent mode. Standard gift sample of Abacavir sulfate and Lamivudine were provided by Aurobindo Ltd., Hyderabad. Distilled water used as a solvent. Stock solution (100µg/ml) of ABAC and LAM prepared by dissolving 10mg of drug in 100ml double distilled water. The maximum absorbance of ABAC and LAM obtained at 284.0nm and 270.0nm respectively. ABAC and LAM shows their respective linearity in the concentration range of 5-30µg/ml at their respective wavelength maxima.

For all two methods, same mixed standard in the linearity range for each drug from 5-30 µg/ml of ABAC and LAM were prepared by diluting appropriate volumes of standard stock solutions. The scanning of solution of ABAC and LAM was carried out in the range of 400nm-200nm.

For Multicomponent analysis using inbuilt software instrument, two sampling wavelength 284.0nm and 270.0nm were selected for the estimation of two drugs. The concentrations of mixed standard solution were entered in the Multicomponent mode. The absorbance spectra of mixed standard and sample solutions were measured at selected wavelength as shown in fig.1. the instrument gives individual concentration of drug present in the sample solution directly.

In the simultaneous equation using AUC method, the 'X' values of each of two drugs were determined at the selected wavelength ranges 256.0-276.0 nm and 271.0-293.0nm. the 'X' values determined as Area under curve of component (from 256.0-276.0nm or 271.0-293.0nm) / concentration of component in g/l ------ (1) A set of two simultaneous equation framed using this 'X' values are as follows:

$A_1 = 677.7C_1 + 277.372C_2$ -------- (2)

$A_2 = 850.16C_1 + 1043.0533C_2$ ---- (3) Where C_1 and C_2 are the concentrations of LAM and ABAC respectively in g/l in sample solution; A_1 and A_2 are the area under curve of sample solution at wavelength range 256.0-276.0nm and 271.0-293.0nm respectively; 677.7 and 277.372 are the 'X' values at wavelength range 256.0-276.0nm of LAM and ABAC respectively; while 850.16 and 1043.0533 are the 'X' values at wavelength range 271.0-293.0nm of LAM and ABAC respectively. The 'X' values reported are the mean of six independent determinations. By applying cramer's rule and matrices in equation (2) and (3), concentrations C_1 and C_2 can be obtained. The absorbance spectra of standard solutions were measured at selected wavelength range as shown in fig.2.

$$C_{lam} = \frac{X_{abac271\text{-}293} \times AUC_{256\text{-}276} - X_{abac256\text{-}276} \times AUC_{271\text{-}293}}{X_{abac271\text{-}293} \times X_{lam256\text{-}276} - X_{abac256\text{-}276} \times X_{lam271\text{-}293}} \quad \text{---------- (4)}$$

$$C_{abac} = \frac{X_{lam256\text{-}276} \times AUC_{271\text{-}293} - X_{lam271\text{-}293} \times AUC_{256\text{-}276}}{X_{abac271\text{-}293} \times X_{lam256\text{-}276} - X_{abac256\text{-}276x} X_{LAM271\text{-}293}} \quad \text{---------- (5)}$$

Where AUC 256-276 and AUC 271-293 are the area under curves of solution at wavelength range between 256-276 nm (LAM) and 271-293 nm (ABAC) respectively.

Twenty tablets of LAM and ABAC in combination were weighed; their average weight was determined and finally crushed to powder sample. from the triturate, tablet powder equivalent to 300mg of LAM and 600 mg of ABAC was weighed and transferred to 100 ml volumetric flask and dissolve in 50 ml water and the content was kept in ultrasonicator for 30 min. finally the volume was made up to the mark with water. The solution was filtered through Whatman filter paper No.41.

This tablet solution was further diluted to obtain 15 µg/ml of LAM and 30µg/ml of ABAC. The mixed sample solution were scanned using proposed methods as discussed above and the results were obtained and reported in table 1. recovery studies were carried out at 80%, 100% and 120% level of the label claim. The percentage recovery of ABAC and LAM in the sample mixture were determined and reported in table 1.

The coefficient correlations were found to be 0.9995 for LAM and 0.9992 for ABAC. The results of tablet analysis and recovery

studies obtained by proposed methods were validated by statistical evaluation. The percentage of coefficient of variation for both the drugs was found to be less than 2%. All the developed methods were found to be simple, rapid, precise, economical and accurate for routine simultaneous estimation of ABAC and LAM in tablet dosage form. The recovery was close to 100% indicating the reproducibility and accuracy of the methods. The Multicomponent method is rapid and easy because it does not require manual calculations. other method like AUC is simple and economical but require few simple calculations.

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TABLE 1: RESULTS OF TABLET ANALYSIS

Method	Tablet sample	Label claim (mg/tab)		Label claim(%)		S.D*		% mean recovery*	
		ABAC	LAM	ABAC	LAM	ABAC	LAM	ABAC	LAM
A	T_1	600	300	100.09	100.26	0.028	0.033	99.99	100.03
B	T_2	600	300	99.97	99.86	0.023	0.015	99.99	99.97

A is Multicomponent method and B is AUC method. T1 is the brand of tablet formulation. * denotes n=6 , average of estimation. ABAC and LAM denotes Abacavir and Lamivudine respectively.

TABLE 2: RECOVERY STUDY DATA FOR TABLET FORMULATION

Method	Level of recovery	% mean recovery		S.D		C.V		S.E	
		ABAC	LAM	ABAC	LAM	ABAC	LAM	ABAC	LAM
A	80% 100% 120%	99.93 100.04 99.97	99.73 100.11 99.83	0.082 0.097 0.034	0.031 0.049 0.150	0.082 0.097 1 0.15	0.031 0.049 5 0.0341	0.033 0.03 0.13	0.12 0.10 0.03
B	80% 100% 120%	99.98 100.11 1	99.95 99.97 100.04	0.036 0.023 0.049	0.025 0.024 0.097	0.036 1 0.023 0.049 52	0.025 1 0.024 2 0.0971	0.15 0.101 0.01	0.11 0.1 0.012

A is Multicomponent method and B is AUC method.* denotes n=6 , average of six estimation. ABAC and LAM denotes Abacavir and Lamivudine respectively.

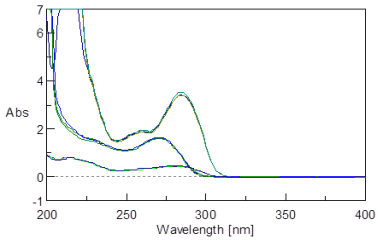

Fig 1. Over lain spectra of mixed standard of ABAC and LAM for Multicomponent method

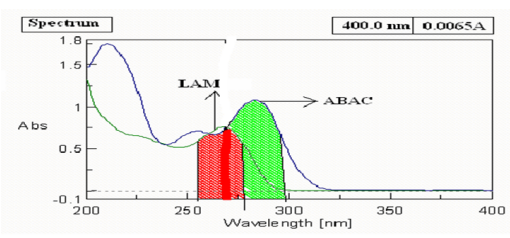

Fig 2. Overlain spectra of standard solutions of ABAC and LAM for AUC method

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