Concurrent quantitative analysis of Abacavir Sulfate and Lamivudine in Combined Dosage Form using First Derivative Zero-Crossing and Ratio First Derivative Spectrophotometric Techniques

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ABSTRACT

Two simple, sensitive, specific and accurate spectrophotometric methods are proposed in the present work for the simultaneous estimation of Abacavir sulfate (ABA) and Lamivudine (LMV) in their binary mixture. In first derivative zero crossing technique the absorbances at 234.2 nm (zero crossing point of LMV) were plotted against the respective concentrations of ABA. Similarly the absorbances at 287.3 nm (zero crossing point of ABA) were plotted against the respective concentrations of LMV. For the ratio spectra first derivative spectrophotometric method, the graphical treatment of the overlapping spectra depends on division of the absorption spectrum of the binary mixture by a standard spectrum of one of the components and then the first derivative amplitudes measured at 233.0nm for ABA and 236.4nm for LMV .The calibration curves follow Beer's law in the range of 5 to 30 μg/ml for ABA and 2.5 to 15 μg/ml for LMV for both the methods. The methods were successfully applied to quantify ABA combined with LMV in tablets and validated accordingly.

Keywords: Abacavir Sulfate; Lamivudine; First derivative zero crossing; Ratio spectra first derivative spectrophotometry.

INTRODUCTION

Chemically Abacavir Sulfate (ABA), is a 1S, 4R [2-Amino,6-(cyclopropylamino)-9H- purin-9-yl]-2- cyclopentene-1-methanol hemisulfate, an antiretroviral drug, which is nucleoside reverse transcriptase inhibitor. The typical dose of ABA is 300 mg per day. Lamivudine (LMV), chemically (2*R*,*cis*)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, is also a nucleoside reverse transcriptase inhibitor. The dose of LMV is 150 mg per day[1-3]. Literature survey revealed that HPLC and spectroscopic methods have been reported for determination of ABA in combination with other drugs[4-6]. Similarly for LMV, literature survey revealed that HPLC, HPTLC and spectroscopic methods have been reported for its determination individually and in combination with other drugs[6-13]. A combination of drugs, ABA (300 mg) and LMV (150 mg) in tablet formulation is

commercially available as a tablet (Epzicom, Glaxosmithkline Ltd., Mumbai, India). To our knowledge no spectrophotometric method has been reported yet for the simultaneous estimation of ABA and LMV. Hence, an attempt has been made to develop and validate, in accordance with ICH guidelines, simple, precise, accurate and economical spectrophotometric methods for quantitative analysis of ABA combined with LMV in tablets respectively. Figure 1 shows the zero order spectra of ABA and LMV and their mixture in methanol. It can be seen that the absorption spectra of ABA and LMV are extensively overlapped and as a result the determination of these two compounds is not possible for reliable direct absorbance measurement.
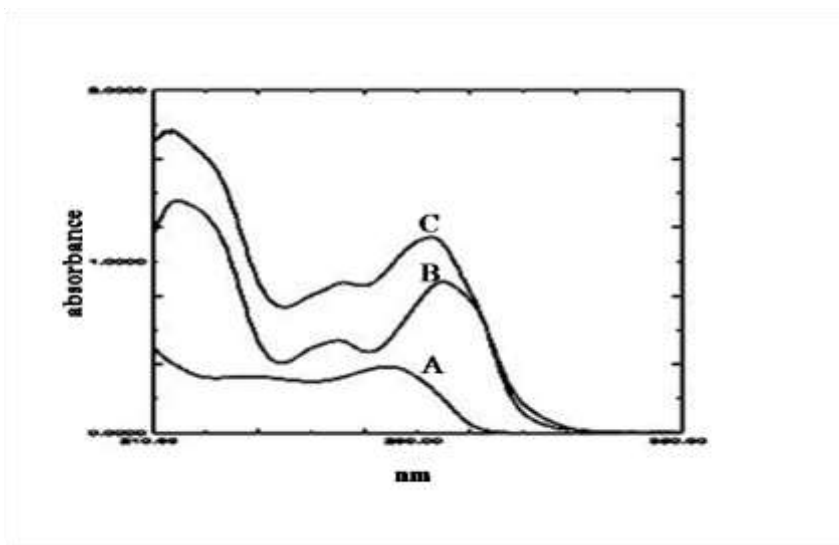
Fig. 1. Zero order overlain spectra of A :-Lamivudine (5μg/ml) B :- Abacavir sulfate (10 μg/ml) C :- Binary mixture of Lamivudine (5μg/ml) and Abacavir sulfate (10 μg/ml) in methanol.

In our study, first derivative zero crossing and first derivative ratio spectra methods [14-16] were applied for the simultaneous analysis of the binary mixtures and a commercial tablet formulation containing ABA and LMV as the derivative methods allows for selection of defined analytical wavelengths of highest value due to the presence of lot of maxima and minima and offers a potential greater sensitivity and accuracy.

EXPERIMENTAL

Instrument:

Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm.

Reagents and Chemicals:

Abacavir sulfate and Lamivudine (Bulk drugs) were kindly provided by Matrix Lab, Nashik and were used as received. Methanol AR grade (Spectrochem Pvt. Ltd., Mumbai,
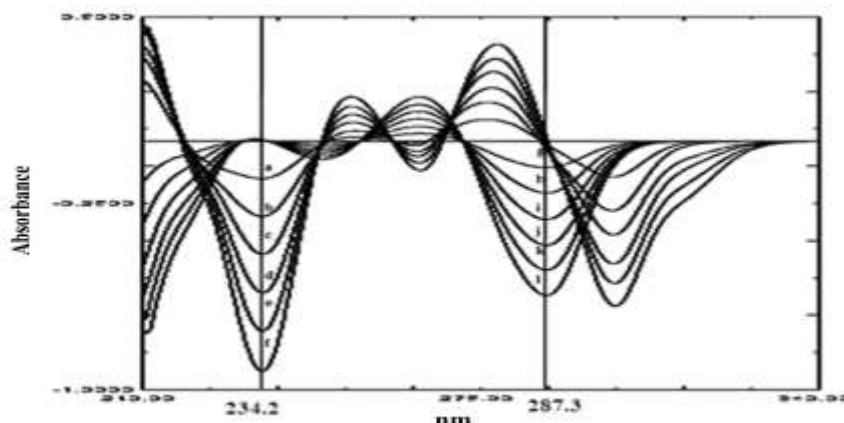
INDIA) was used in the spectrophotometric analysis. The formulation Epzicom was purchased from local market.

Preparation of standard solutions and binary mixture:

Individual standard stock solutions of ABA (100 µg/ml) and LMV (100 µg/ml) were prepared by dissolving 11.72 mg ABA (equivalent to 10 mg Abacavir) and 10.0 mg LMV in 100 mL methanol. The standard stock solutions were further diluted separately to obtain working standard solutions of concentration 5-30 µg/ml of ABA and 2.5-15 µg/ml of LMV. Similarly accurate volumes of each of ABA and LMV stock solutions were transferred in 10ml volumetric flasks and diluted to volume with methanol to obtain binary mixtures within the linearity range for the both drugs.

First derivative zero-crossing method (Method A):

The first derivative of the standard solutions were obtained by using smoothing factor $\Delta\lambda = 8$ and scaling factor 10 for determining zero cross points for both the drugs. The peak amplitude of first derivative spectra was measured at 234.2 nm (zero crossing point of LMV) and 287.3 nm (zero crossing point of ABA) for ABA and LMV, respectively as shown in Figure 2.

Fig.2. First derivative spectra of ABA (a) 5 µg/ml (b)10 µg/ml (c) 15 µg/ml (d) 20 µg/ml (e) 25 µg/ml (f) 30 µg/ml at 234.2nm (zero crossing of LMV) and LMV (g) 2.5 µg/ml (h)5 µg/ml (i) 7.5 µg/ml (j) 10 µg/ml (k) 12.5 µg/ml (l) 15 µg/ml at 287.3nm (zero crossing of ABA).

To optimize the simultaneous determination of ABA and LMV by using first derivative zero crossing method, it was necessary to test the influence of the variables like derivative intervals ($\Delta\lambda$) and scaling factor on derivative spectra. Derivative intervals ($\Delta\lambda$) parameter needs to be optimised to give a good selectivity and a higher sensitivity and an adequate signal-to-noise ratio. Various values of $\Delta\lambda$ were tested, a value of $\Delta\lambda=8$ was found optimal in connection with both slit width and wavelength interval. The other parameter affecting the shape of derivative spectra is scaling factor. In this method optimised value of scaling factor is 10.

First derivative ratio spectra method (Method B):

The absorption spectra of the solutions prepared at different concentrations of ABA and of its binary mixtures with LMV were recorded and divided wavelength-by-wavelength by the spectrum of the standard solution of LMV (10 μg/ml). Then the first derivatives of the ratio spectra were calculated with $\Delta\lambda=8$nm and scaling factor 10. In the binary mixtures the amount of ABA was determined by measuring the first derivative signal at 233.0nm. A similar procedure was followed for the different concentrations of LMV and its binary mixtures with ABA, where ABA (10 μg/ml) used as a divisor. In the same way as described above, the content of LMV was determined by selecting the first derivative of the ratio spectrum signal at 236.4 nm. To optimize the simultaneous determination of ABA and LMV by using ratio spectra derivative spectrophotometric method, it was necessary to test the influence of the variables like the standard divisor concentration, derivative intervals ($\Delta\lambda$) and scaling factor on derivative ratio spectra.

A- Effect of standard divisor concentration.

In a preliminary investigation, for selecting the standard solution as divisor, appropriate concentration of ABA and LMV 5-30 μg/ml and 2.5-15 μg/ml were tested respectively. Stored spectra of standard ABA solutions were divided wavelength by wavelength by a standard spectrum of LMV ranging from 2.5-15μg/ml as shown in Table 1.

Table 1. Optimization of divisor concentration for Abacavir sulfate.

Compound	LMV Divisor Conc. (μg/mL)	λ (nm)	R^2
Abacavir sulfate (5-30 μg/mL)	2.5	233	0.9819
	5	233	0.9989
	7.5	233	0.9983
	10	232.8	0.9998
	12.5	232.8	0.939

Then the first derivative of above ratio spectra was recorded and the values of the derivatives were measured at suitably selected wavelengths and plotting against the corresponding concentration to obtain the calibration graph. The similar procedure was followed for the different concentration of LMV when ABA 5-30μg/ml was used as divisor in the same way as shown in Table 2.

Table 2. Optimization of divisor concentration for Lamivudine.

Compound	ABV Divisor Conc. (μg/mL)	λ (nm)	R^2
Lamivudine (2.5-15 μg/mL)	5	236.2	0.9991
	10	236.4	0.9997
	15	236.8	0.9992
	20	236.8	0.9885
	25	237	0.9980

The calibration curve was obtained by plotting absorbance versus drug concentration. Thus a concentration of 10μg/ml of ABA and 10μg/ml of LMV as divisor gave best results in terms of signal to noise ratio and highest correlation coefficient values, being an indication of the quality of fitting of the data to the straight line.

B-Effects of derivative intervals (Δλ) and scaling factor on derivative ratio spectra.

Δλ, the width of the boundaries over which the derivative is calculated was tested for Δλ=2nm, 4nm, 8nm and 16nm. The value of Δλ=8nm was found optimal in connection with both slit width and wavelength interval.

Thus the absorption spectra of ABA for the solution in methanol were divided by the spectrum of the standard solution of 10 μg/ml of LMV in the same solvent ,then first derivative was plotted with the interval of Δλ=8nm and scaling factor=10 as shown in Figure 3A and Figure 3B . It was found that the first derivative amplitude at 233.0 nm was suitable for the determination of ABA in binary mixture with LMV.
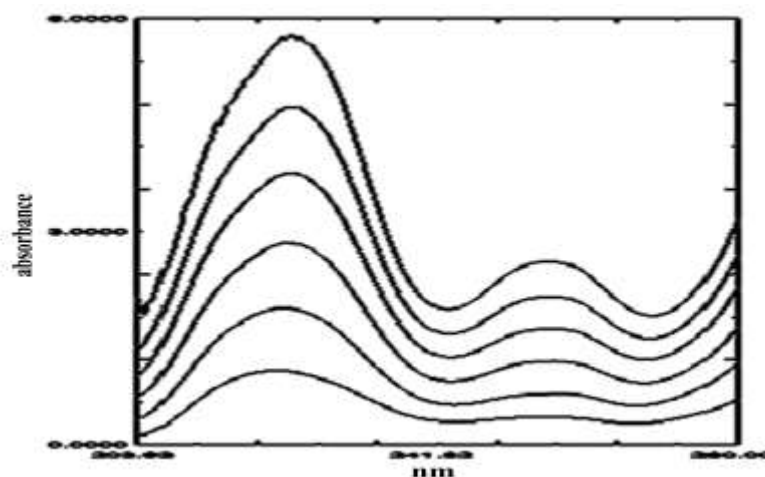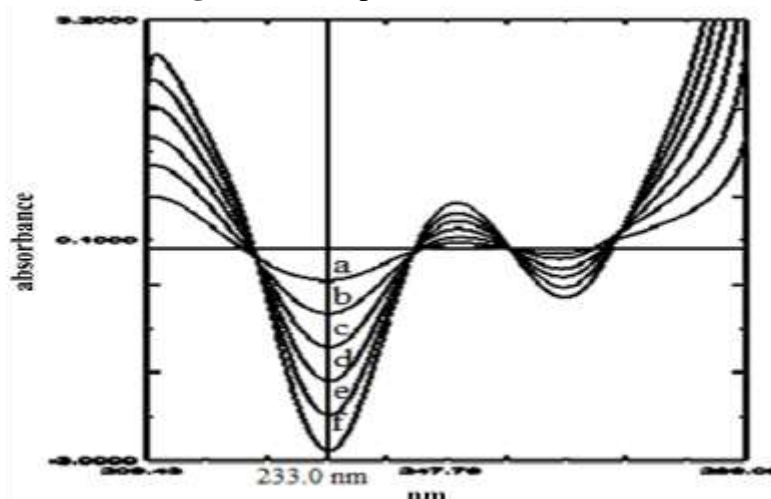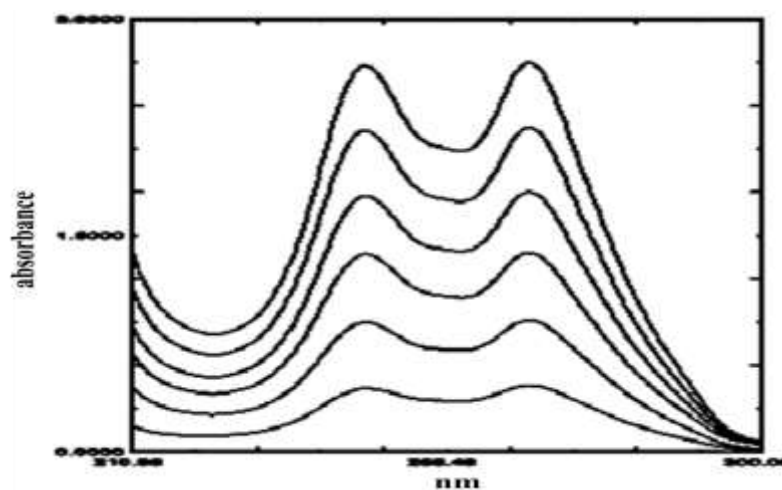

Fig.3A. Ratio spectra of ABA

Fig.3B. First derivative of the ratio spectra of ABA. (a) 5 μg/ml (b)10 μg/ml (c) 15 μg/ml (d) 20 μg/ml (e) 25 μg/ml (f) 30 μg/ml using 10 μg/ml LMV as divisor in methanol (Δλ=8 nm).

In similar way, the stored spectra of LMV were divided wavelength by wavelength, by 10 μg/ml of ABA, then first derivative was plotted with the interval of Δλ=8 nm and

scaling factor=10.as shown in Figure 4A and 4B. It was found that the first derivative amplitude at 236.4 nm was suitable for the determination of LMV in binary mixture with ABA.

Once the optimum working condition was established, calibration graphs were obtained at 233.0 nm for Abacavir sulfate and 236.4 nm for Lamivudine in the standard binary mixture and showed that the proposed method is applicable over the ranges 5-30 µg/ml for ABA and 2.5-15 µg/ml for LMV.

Fig.4A. Ratio spectra of LMV
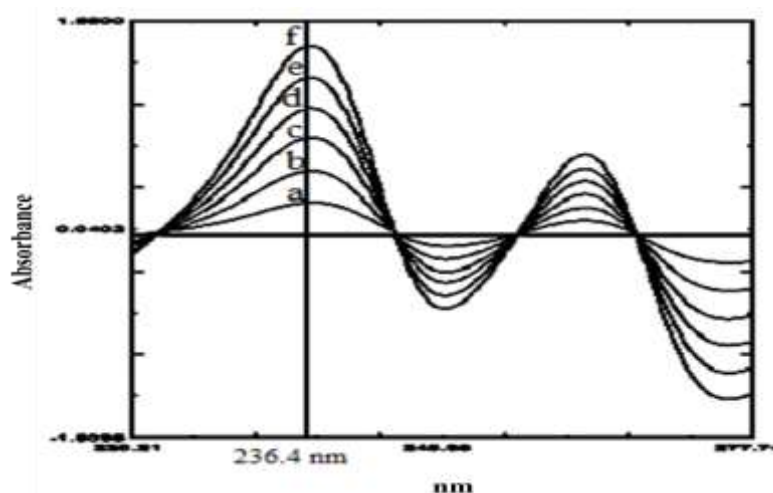
Fig.4B. First derivative of the ratio spectra of LMV. (a) 2.5 µg/ml (b)5 µg/ml (c) 7.5 µg/ml (d) 10 µg/ml (e) 12.5 µg/ml (f) 15 µg/ml using 10 µg/ml ABA as divisor in methanol (Δλ=8 nm).

RESULTS AND DISCUSSION

The proposed methods gave successful results for the quantitative determination of the two component mixtures and a commercial tablet formulation consisting of two compounds.

Validation of developed methods:

To determine the validity and applicability of the developed method, recovery studies were performed in the synthetic mixtures of ABA and LMV with different ratios. The characteristic parameters of the regression equations with LOD and LOQ and the results of recovery studies are complied in Table 3 and Table 4 respectively.

Table 3. Validation parameters

Parameters	Method A		Method B	
	ABA	**LMV**	**ABA**	**LMV**
Slope	0.0345	0.0215	0.108	0.0681
Intercept	0.0136	0.0025	0.0476	0.0128
Correlation coefficient	0.9995	0.9997	0.9995	0.9998
Linearity range(µg/ml)	5.0- 30	2.5-15	5.0 – 30	2.5-15
LOD (µg/ml)	0.10	0.13	0.03	0.05
LOQ (µg/ml)	0.30	0.39	0.10	0.14
Precision (% RSD)				
A. Intra-day (n=3)	0.77	0.67	0.63	0.18
B. Inter-day (n=9)	0.79	0.69	0.95	0.76
Reproducibility (%RSD)	< 2%	< 2%	< 2%	< 2%

% RSD is percentage relative standard deviation

Table 4: Results of the recovery studies

Method	Spiked level (µg/ml)		Percent recovery % ± SD (n= 5)	
	ABA	**LMV**	**ABA**	**LMV**
A	8	4	99.90±0.289	98.76±0.124
B	8	4	99.97±0.411	99.97±0.289
A	10	5	99.37±0.321	99.15±0.123
B	10	5	99.68±0.126	100.23±0.226
A	12	6	99.83±0.201	99.32±0.041
B	12	6	100.07±0.384	100.31±0.147

Procedure for analysis of tablet formulation:

A total of 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet (containing 300 mg of ABA and 150 mg of LMV) was taken and dissolved in 50 ml methanol by sonicating it for 10 minutes. Then it was transferred to a 100 ml volumetric flask through a Whatman No. 40 filter paper. The residue was washed thrice with methanol and the combined filtrate was made up to the mark with methanol. The sample solution thus prepared was diluted with methanol to get the solutions containing ABA and LMV in 10:5 µg ml^{-1} proportion respectively. The absorbance of sample solutions were measured at selected wavelengths and the concentrations of the two drugs were estimated. The analysis procedure was repeated six times and the results are depicted in Table 5.

Table 5. Results of the assay in commercial tablet formulation

Tablet	Method	%Recovery ± SD	
		ABA	**LMV**
Epzicom	A (n=6)	99.63±1.40	99.84.±1.02
	B (n=6)	100.05.±0.92	99.89.±1.28

CONCLUSION

The results of the proposed methods were found to be very close to the labeled value of commercial pharmaceutical formulation. This can be considered as an advantage of the investigated methods for the quantitative resolution of the two component mixtures. The procedures are fast and specific and work without solving equations or separation steps. Thus the developed methods can be strongly applied to a routine analysis, quality control of binary mixtures, and commercial products containing these two drugs.

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