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A NOVEL RATIO SPECTRA DERIVATIVE APPROACH FOR DETERMINATION OF RIVAROXABAN AND ASPIRIN IN COMBINED CARDIOVASCULAR DOSAGE FORMS

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Abstract: In the present work, the ratio spectra first derivative spectrophotometry is proposed for the resolution of Rivaroxaban (RIVA) and Aspirin (ASP) in synthetic mixtures prepared in 1:10 ratio. The calibration graph follows Beer's law in the range of 5 to 25 µg/ml for rivaroxaban and from 50-250µg/ml for aspirin. The mean recoveries & relative standard deviations were found as 100.03% & 0.273% for RIVA and 100.02% and 0.144% for ASP, respectively in 1:10 ratio. 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 calculating the first derivative of the ratio spectrum. These approaches were successfully applied to quantify RIVA combined with ASP in tablets respectively and validated the ratio in synthetic mixtures.

Keywords: Rivaroxaban, Aspirin, Ratio spectra, Overlapping spectra, Beer's law.

Introduction:

Rivaroxaban (RIVA) 5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1,3-oxazolidin-5-yl}methyl) thiophene-2-carboxamide**.** Rivaroxaban is a factor Xa inhibitor used to treat deep vein thrombosis (DVT) and pulmonary embolism (PE). Aspirin (ASP), acetyl salicylic acid[2] is a non steroidal anti inflammatory drug having analgesic and antipyretic activity with an effective inhibition of platelet aggregation. Rivaroxaban, chemically known as 5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide,has a molecular formula of C19H18ClN3O5S and acts as an oral anticoagulant by directly inhibiting Factor Xa, a crucial enzyme in the blood coagulation cascade. Aspirin, or acetylsalicylic acid, with a molecular formula of C9H8O4, is a widely used NSAID known for its anti-inflammatory and antiplatelet effects. In UV spectrophotometric analysis. Accurate quantification of both drugs in bulk and combination formulations is essential. UV spectrophotometry offers a simple, cost-effective method for such analysis[1]. The developed UV method is simple, accurate, and validated. It can be effectively

used for routine analysis of rivaroxaban and aspirin in pharmaceutical formulations. Several methods appear in the literature for the simultaneous determination of RIVA and ASP in their binary mixture based on spectrophotometry [2-4], HPLC[5-7], and HPTLC[8]. To our knowledge there is no official method for the simultaneous determination of these drugs in any pharmacopoeia. The resolution of binary mixtures of compounds with overlapped spectra by derivative technique is frequently made on the basis of zero crossing measurements. However the zero order derivative technique cannot be satisfactorily performed because of the large overlap of the absorption spectra as is occurring in the case of RIVA and ASP. Hence a ratio spectra first derivative spectrophotometric procedure has been developed for its better resolution. The aim of present study was to demonstrate the capability of the ratio spectra derivative spectrophotometric method to resolve and overcome the problem of overlapping spectral bands and allow the simultaneous determination of RIVA and ASP without any chemical separation.

Experimental:

Apparatus: Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu U 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:

RIVA were kindly supplied as gift samples by Megafine (Mumbai, India) and ASP were supplied as gift sample by Wockhardt research centre, (Aurangabad, India) were used as received.Analytical grade methanol was used as a solvent.

Standard Solutions:

Stock solution of 5mg/100ml RIVA and 10mg/10ml ASP were prepared separately in methanol. The standard series of the solutions containing 5-25 µg/ml RIVA and 50-250 µg/ml were prepared from the stock solutions for obtaining calibration graphs and spectra. All the solutions were prepared freshly.

Tablet Formulation:

Sample Solution:

Twenty tablets were accurately weighed and powdered in a mortar. An accurately weighed amount equivalent to one tablet was dissolved in methanol in a 100ml calibrated volumetric flask. After 30minutes of mechanical shaking the solution was filtered through what man no.42 filter paper. Appropriate dilutions were prepared in methanol taking suitable aliquots of the clear filtrates.

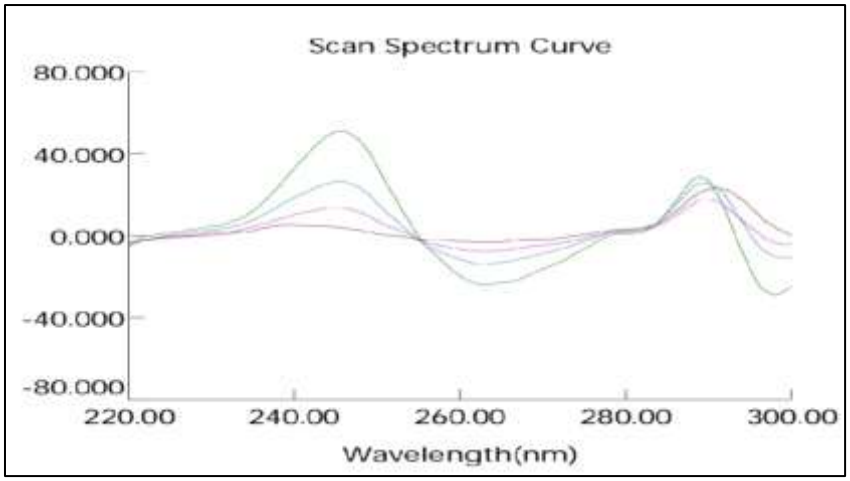

Figure 1: Ratio spectra of RIVA

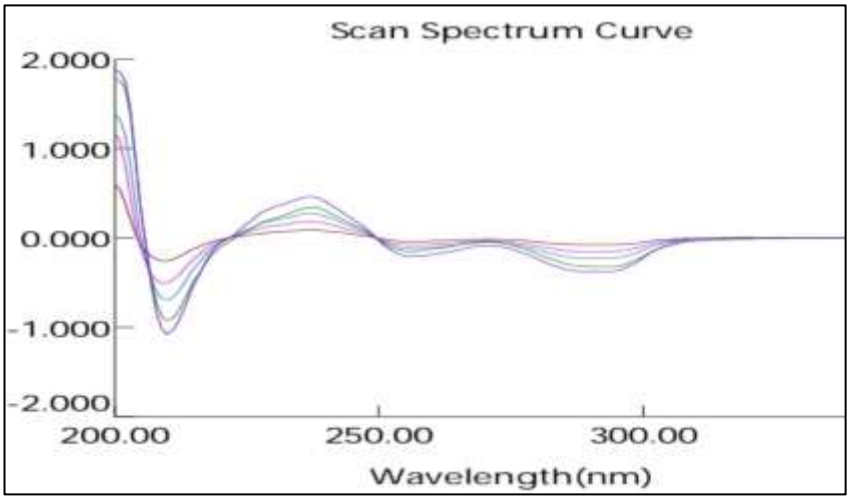

Figure 2: Ratio derivative spectra of RIVA

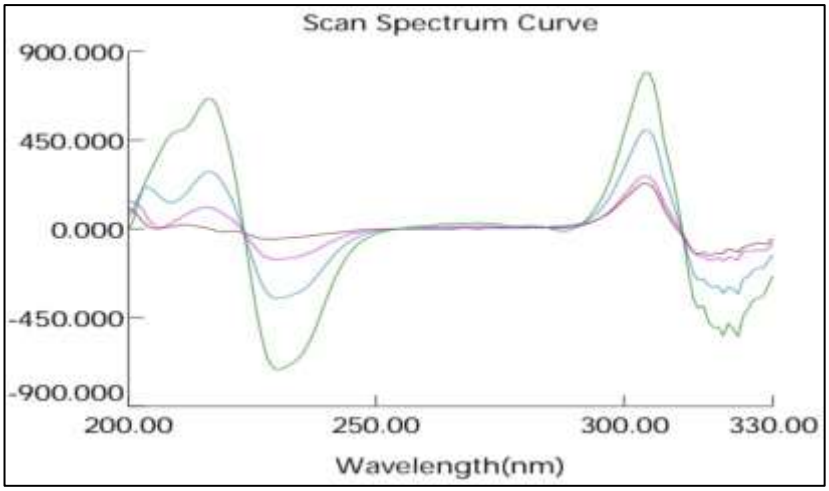

Figure 3: Ratio spectra of ASP

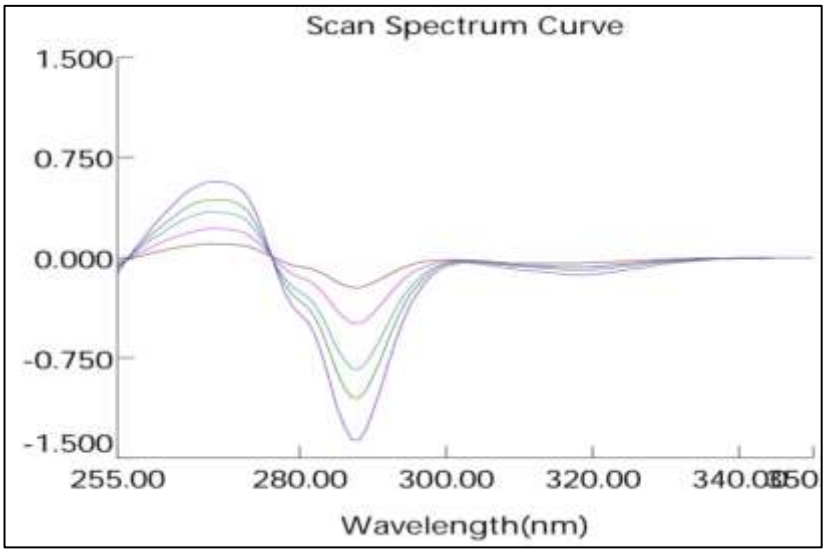

Figure 4: Ratio derivative spectra of ASP

Result and Discussion:

"RIVAandASP and their mixture in methanol can be seen that the absorption spectra of RIVAandASP are extensively overlapped and as a result the determination of these two compounds is not possible for reliable direct absorbance measurement. The main disadvantage of zero crossing derivative spectrophotometry method for resolution of binary mixture is that the selection of analytical wavelengths is critical due to very small distance between them and the values obtained are also small. Hence ratio derivative spectrophotometric method can be suitable to obviate this problem. The method allows for selection of defined analytical wavelengths of highest value due to presence of lot of maxima and minima. Hence the method offers a potential greater sensitivity and accuracy.

Optimization of parameters[9-10]: To optimize the simultaneous determination of RIVA and ASP by using ratio spectra derivative spectrophotometric method, it is necessary to test the influence of the variables like:

1) Effect of standard divisor concentration:

In a preliminary investigation, for selecting the standard solution as divisor, appropriate concentration of RIVA and ASP in the range 5- 25µg/ml and 50-250µg/ml were tested respectively. Stored spectra of standard Rivaroxaban solutions were divided wavelength by wavelength by a standard spectra of ASP (5-25µg/ml) Table 1. Then the first derivative of above ratio spectra were 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

ASP when Riva (5-25µg/ml) was used as divisor in the same way as described above. Table 2. The calibration curve was obtained by plotting absorbance versus drug concentration. Thus a concentration of 50µg/ml of RIVA and 10µg/ml of ASP as a divisor gave best results in terms of signal to noise ratio and correlation coefficient values, being an indication of quality of fitting of the data to straight line.

2) Effects of derivative intervals Δλ on derivative ratio spectra:

Δλ, the width of the boundaries over which the derivative is calculated was tested for Δλ=2nm, 4nm and 8nm. The value of Δλ=4nm was found optimal in connection with both slit width and wavelength interval. Thus the absorption spectra of RIVA for the solution in methanol were divided bythe spectrum of the standard solution of 50µg/ml of ASP in the same solvent (Figure.2a) and the first derivative was plotted with the interval of Δλ=4 nm. (Figure2b). It was found that the first derivative amplitudes at 245.7 nm and 263.2 nm were suitable for the determination of RIVA in binary mixture with ASP. In similar way, the stored spectra of ASP weredividedwavelengthbywavelength,by10µg/ml of RIVA(Figure3a).

Table 1: Optimization of divisor concentration for Rivaroxaban.

Compound	**Divisor conc**	**λnm**	**R^2**
Rivaroxaban (5-25µg/ml)	**50A**	**245nm**	**0.9995**
	100A	245nm	0.9985
	150A	245nm	0.9976
	200A	245nm	0.9984
	250A	245nm	0.9986

Table 2: Optimization of divisor concentration for Aspirin.

Compound	**Divisor conc**	**λnm**	**R^2**
Aspirinn (50-250µg/ml)	5R	304nm	0.9989
	10R	**304nm**	**0.9997**
	15R	304nm	0.9991
	20R	304nm	0.9982
	25R	304nm	0.9988

First derivative was plotted with the interval of Δλ=4nm (Figure 3b). It was found that the first derivative amplitude at 304.4 nm was suitable for the determination of ASP in

binary mixture with RIVA. Once the optimum working condition was established, calibration graphs were obtained at 250nm for Rivaroxaban and 276nm for Aspirin in the standard binary mixture and showed that the proposed method is applicable over the ranges 5-25 µg/ml for RIVA and 50-250 µg/ml for ASP. The characteristic parameters of the regression equations with LOD and LOQ are compiled in Table 3.

Table 3: Analytical data of the calibration graphs for the determination of Rivaroxaban and Aspirin

Parameters	Rivaroxaban	Aspirin
Wavelength(nm)	250	276
Beer'sLawlimit (µg/ml)	5-25	50-250
LOD(µg/ml)	0.077	0.45
LOQ(µg/ml)	0.25	0.084
Intercept	0.009	1.4425
Slope	0.0487	0.074
Correlationcoefficient	0.9994	0.9998

To determine the validity and applicability of the developed method, recovery studies were performed in the synthetic mixtures of RIVA and ASP. Mean recoveries and %RSD were calculated (Table 4).

Table 4: Replicate determinations of synthetic mixtures of RIVA and ASP

a) Analysis of Rivaroxaban in synthetic mixture:

Levels	Test Amount (µg/ml)	Amount of drug spiked	Total amount recovered	±SD	%RSD	%Recovery	Mean
	10	8	28.93			100.28%	
80%	10	8	28.99	0.0503	0.174%	99.86%	100.04
	10	8	28.99			100.00%	
	10	10	32.17			99.87%	
100%	10	10	32.21	0.0200	0.062%	100.00%	99.96
	10	10	32.21			100.00%	

	10	12	35.48			99.82%	
120%	10	12	35.52	0.0490	0.138%	100.00%	100.00
	10	12	35.52			100.18%	
Mean	100.00%						
±SD	0.039						
%RSD	0.124						

b) Analysis of Aspirin in synthetic mixture:

Levels	Test Amount (µg/ml)	Amount of drug spiked	Total amount recovered	±SD	%RSD	%Recovery	Mean
	10	8	174.12			99.85%	
80%	10	8	174.39	0.127	0.073%	100.00%	99.97
	10	8	174.39			100.08%	
	10	10	205.47			100.13%	
100%	10	10	205.57	0.055	0.027%	99.93%	100.02
	10	10	205.60			100.00%	
	10	12	215.60			100.06%	
120%	10	12	215.63	0.021	0.010%	99.94%	100.00
	10	12	215.65			100.00%	
Mean	99.98%						
±SD	0.067						
%RSD	0.037						

Aspirin in the standard binary mixture and showed that the proposed method is applicable over the ranges 5-25 µg/ml for RIVA and 50-250 µg/ml for ASP. The characteristic parameters of the regression equations with LOD and LOQ are compiled in Table 3.

To determine the validity and applicability of the developed method, recovery studies 13-18 wereperformed in the synthetic mixtures of RIVA and ASP with different ratios. Mean recoveries and %RSD were calculated. (Table 4)[16-23]

Conclusion:

An accurate, precise, and robust Ratio Spectra Derivative UV spectrophotometric method was successfully developed and validated for the simultaneous estimation of Rivaroxaban

and Aspirin in bulk and combined dosage form. The method effectively resolved the overlapping spectra of the two drugs without prior separation, enabling their quantification in a cost-effective and time-efficient manner. Validation of the method was carried out as per ICH guidelines, demonstrating excellent linearity, accuracy, precision, and specificity. This analytical approach is suitable for routine quality control analysis of Rivaroxaban and Aspirin in pharmaceutical formulations.

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