Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL 11, ISSUE 10, 2020

CHEMOMETRIC ANALYSIS FOR CONCURRENT QUANTIFICATION OF RIVAROXABAN AND ASPIRIN IN CARDIOVASCULAR FIXED-DOSE COMBINATIONS

Dr. Dipti B. Ruikar[1], Deepak S. Mohale[2], Mr.Gajanan J. Deshmukh[3], Achal H. Yelane[4],Rutwik D. Talokar[4]

[1*] School of Pharmacy G.H Raisoni University, Amravati
[2] Pataldhamal Wadhwani College of Pharmacy, Yawatmal
[3] Department of Pharmacy, Government Polytechnic, Amravati, Maharashtra
[4] P. R. Pote Patil College of Pharmacy, Amravati, 444601, Maharashtra, India

***Corresponding author: Dr. Dipti B.Ruikar**
dipti21ruikar@gmail.com

Abstract:

Rivaroxaban, a factor Xa inhibitor, treats deep vein thrombosis and pulmonary embolism. Aspirin, an antithrombotic agent, is used to prevent and manage strokes and other cardiovascular issues. Accurate analytical methods are needed to analyze both drugs.

Chemometric methods of analysis (Inverse Least Square&Classical Least Square) were applied to the simultaneous assay of Rivaroxaban & Aspirin in their synthetic mixture. 12 mixed solutions were prepared for the chemometric calibration as training set & 10 mixed solutions were prepared as validation set. The absorbance data matrix was obtained by measuring the absorbance at 21 wavelength points, from 240-340nm with an interval of 5nm. Linearity was observed in the concentration range of 5-30mcg/ml for Rivaroxaban and 50-300mcg/ml for Aspirin using methanol as a solvent. The accuracy of the methods was assessed by recovery studies and was found to be within the range of 98-102%for both drugs. The % RSD value were found to be less than 2, proving the method were precise. RMSEP is used for examining the errors in the predicted concentrations. The methods were compared using ANOVA, f-test, and t-test. The results were validated statistically as per ICH (Q2) R1 guideline and were found to be satisfactory.

Keywords: Chemometric method of analysis, Classical least square, Inverse least square, Statistical analysis, Anticoagulant, Antiplatelets**.**

Introduction

To determine the component concentration in the unknown sample. For both classical statistics and chemometric approaches, there is currently a considerable amount of computer software readily available. Chemometric methods are particularly useful approaches for

analysing many compounds' spectra generating an interference that makes determining the amounts of each component impossible. In addition, chemometrics calibration methods are simple since they can evaluate a large number of samples in a short amount of time more accurately and precisely compared with other methods it can be described as the use of mathematical and statistical approaches to create and/ or optimize measurement procedure as well as the analysis of pertinent data to offer chemical information. multivariate calibration such as classical least square&inverseleast square have been widely used in quantitative spectrum analysis in recent years to extract selective information from unselective data. CLS & ILS are two of the most basic approaches, both based on Beer's principle and using a multivariate least square procedure.[1]

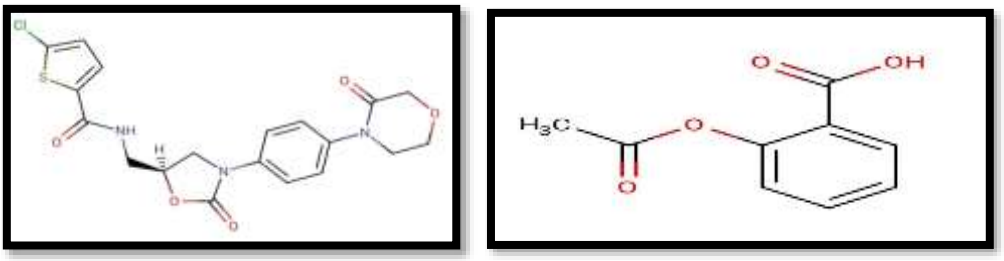
Aspirin, known chemically as 2-(acetyloxy) benzoic acid, is a cyclooxygenase inhibitor that is widely recognized for its antiplatelet properties. It appears as a white Aspirin, chemically identified as 2-(acetyloxy) benzoic acid, acts as a cyclooxygenase inhibitor, and is well-known for its antiplatelet effects. It is a white crystalline powder with weak acidity, having a molecular mass of 180.157 g/mol and the molecular formula C9H8O4 (see Fig 1). Aspirin is a prominent anti-thrombogenic agent extensively used for treating and preventing cerebrovascular and cardiovascular conditions, such as stroke. Antiplatelet therapy with aspirin has been shown to reduce the risk of cardiovascular diseases following acute myocardial infarction, coronary artery bypass grafting, and in patients with chronic atrial fibrillation, among other risk factors [2].

Traditional treatment of these disorders involves the use of heparin, oral anticoagulants, and the preferred antiplatelet agent, aspirin. Interestingly, aspirin was not originally intended to be an antiplatelet drug; however, after being repurposed, it has become one of the most commonly prescribed antithrombotic medications [2].

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, is a small molecule with a molecular weight of 436 g/mol. It is only slightly soluble in organic solvents and is practically insoluble in water. Its molecular formula is C19H18ClN3O5S (see Figure 1).

Rivaroxaban is a novel oral direct Factor Xa inhibitor currently in advanced clinical development for the prevention and treatment of thromboembolic diseases [3]. Approved in over 100 countries, including the European Union and Canada, it is used to prevent venous thromboembolism in adult patients undergoing elective hip or knee replacement surgery. Additionally, rivaroxaban is being developed further for the treatment of thromboembolic

disorders, stroke prevention in patients with atrial fibrillation, and the secondary prevention of acute coronary syndromes. Uniquely, rivaroxaban specifically targets Factor Xa within the coagulation cascade and operates independently of antithrombin [4]. The US Food and Drug Administration (FDA) has approved the use of Rivaroxaban in combination with Aspirin to lower the risk of major cardiovascular (CV) events in adults with chronic coronary artery disease (CAD) or peripheral artery disease (PAD) [5].



Rivaroxaban **Aspirin**

Fig.1. Chemical structures of Rivaroxaban and Aspirin

Experimental work:

Selection of common solvent:

Reported solubility of Rivaroxaban & Aspirinwas found to be in methanol. Hence method was chosen as a common solvent for the simultaneous estimation of both the drugs.

Chemicals and reagents:

- Rivaroxaban & Aspirin(Bulk drugs) were kindly provided by 'Megafine, Mumbai and Wockhart Research center MIDC Aurangabad" resp. were used as received.
- Methanol used in the spectrophotometric analysis was of analytical reagent grade.

Instrument:

A Lab India double beam spectrophotometer with a fixed slit width (1nm) was used, coupled with UV Win spectrophotometer software. The numerical calculations were performed using MATLAB 6.1 SOFTWARE & EXCEL.

Light absorption study:

In order to develop UV spectrophotometric method for simultaneous estimation of RIVA and ASP, light absorption study was done. Individual spectrum of both the drugs and their binary mixture was overlaid to obtain suitable wavelengths that could be used for the simultaneous estimation methods.

Preparation of standard stock solution, calibration and validation set:

Stock solution of 100mg/100ml from pure drug samples of Rivaroxaban & Aspirinwere prepared separately in methanol. Standard solution of Rivaroxaban & Aspirincontaining concentration ranges of 5-30μg/ml and 50-350 μg/ml were prepared in methanol, respectively. All the solutions were scanned in the wavelength range of 200-400nm. And graphs were stored in the computer.

Construction of the calibration (training) set [6]

A training set consisting of 12 binary mixture solutions in the possible combinations containing 0-25 μg/ml of Rivaroxaban. & 0-300 μg/ml of Aspirin was used for Chemometric Calibrations as illustrated inTable 1. The zero order absorbance spectra were measured & stored in the computer. To estimate the ILS & CLS models for the training set, the computer was fed with absorbance & concentration matrices, then calculations were carried out with the use of proposed software.

Table 1: Composition of Calibration (Training) set for both the drugs used in ILS techniques:

Mixture no.	Rivaroxaban	Aspirin
1	5	150
2	10	150
3	15	150
4	20	150
5	25	150
6	15	0
7	15	50
8	15	100
9	15	200
10	15	250
11	15	300
12	0	150

Construction of the validation set [7-9]:

Different mixtures of the two drugs were prepared by diluting different volumes of Rivaroxaban & Aspirin. standard solutions in 10 ml measuring flask & diluting to volume with methanol. (Table 2).

Table 2: Composition of Validation set for both the drugs used in ILS techniques:

Mixture no.	Rivaroxaban	Aspirin
1	10	100

2	15	100
3	20	100
4	25	100
5	30	100
6	10	150
7	10	200
8	10	250
9	10	300
10	10	350

Inverse least square

It is also called as P-matrix calibration as it originally requires the use of multiple linear regression to calculate the inverse expression of the Beers-Lambert equation of spectroscopy.

$$C=PA$$

Where, C=concentration matrix

P=calibration coefficient, and

A=absorbance matrix.

To determine P, a training set containing a concentration matrix, C, and an absorbance matrix, A, is used to create a calibration using ILS. ILS differs from the Classical technique, which involves fitting a liner mixture of pure spectra to an unknown spectrum. This distinction provides ILS with several advantages. When all of the system components aren't explicitly evaluated, CLS fails to provide accurate predictions.The MATLAB 6.1 SOFTWARE & EXCEL was used to construct the approach[10]

Classical least square

CLS is also known as the K matrix. It involves the usage of multiple linear regression to represent the Beer-Lambert law of spectroscopy classically.

$$A=KC$$

The calibration set comprised of concentration matrix, C, and an absorbance matrix, A for known sets of samples constructed to generate calibration using CLS. In MATLAB 6.1 SOFTWARE & EXCEL, the CLS model was developed by adding absorbance (A) and concentration matrix (C) data.

The calculated K can be used to forecast the concentration of an unknown sample, Cunk, based on its measured spectrum, and it can be stored as an absorbance matrix, Aunk.

There are mainly two subclasses of CLS namely, direct CLS and indirect CLS. The K matrix is calculated in direct CLS by measuring the spectra of the pure component, either neat or in a nonadsorbing solvent. In the indirect CLS technique, pure spectra are calculated from mixture spectra rather than being measured directly.

Absorbance matrix A is comprised of Zero-order spectra at 5 nm intervals between 240nm to 340nm, that is , absorbance at 21 wavelength points. The developed model comprised absorbance values of sample at 21 wavelength points, and quantities of Rivaroxaban and Aspirin in the validation data set as well as in tablet formulations were predicted.[1]

Results & Discussion:

In the spectral work, the following steps can explain the fundamental concepts of ILS.

Selection of the spectral region

Although ILS are the full spectrum method, 21 wavelengths were selected from 240nm to 340nm with the interval of $\Delta\lambda$ = 5nm in the zero order spectra.

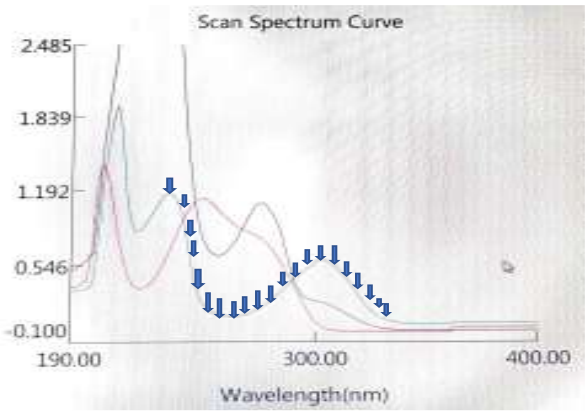


Fig 1: - Overlay spectra of Rivaroxaban, Aspirin and their binary mixture showing spectral region 240nm-340nm (21 wavelengths) [11]

Measurement of the absorbances:

The absorbance matrices were produced by measuring absorbances at 21 wavelengths. In this calibration was obtained by measuring absorbances data matrix & concentration data matrix to predict the concentration of Rivaroxaban & Aspirinin their synthetic mixtures. The numerical calculations were performed using MATLAB 6.1 SOFTWARE & EXCEL **[1]**

Equation for ILS (Inverse Least Square) Method (1):

The mathematical expression **C=P×A** in the matrix is given as

$C_1 = P_{11}A_1 + P_{12}A_2 + \ldots\ldots P_{1w}A_w$

$$C_2 = P_{21}A_1 + P_{22}A_2 + \ldots\ldots P_{2w}A_w$$

$$C_3 = P_{31}A_1 + P_{32}A_2 + \ldots\ldots P_{3w}A_w$$

: : :

: : : :

$$C_c = P_{c1}A_1 + P_{c2}A_2 + \ldots\ldots P_{cw}A_w$$

Where,

A_w = Absorbance at the w^{th} wavelength.

P_{cw} = Calibration coefficient for the c^{th} component at the w^{th} wavelength.

C_c = Concentration of the c^{th} component

In this method, the calibration coefficient (P) was obtained from the linear equation system using the absorbance data & the training set.

The absorbance values of the samples at 21 wavelengths were placed in the above equation & the amounts of Rivaroxaban & Aspirin. in the synthetic mixtures were found as shown in (Table 3).

Introducing (P) into linear equation system.

Introducing P into the linear equation system with an absorbance matrix of sample gave concentration of RIVA and ASP in the sample mixture.

$$\begin{pmatrix} C_{Riva} \\ C_{ASP} \end{pmatrix} = \begin{pmatrix} 0.0448 & -1.8937 \\ -0.1349 & 3.6503 \\ 0.0960 & -0.2636 \\ 0.1167 & -1.2676 \\ -0.1364 & -1.4340 \\ -0.0126 & 0.8620 \\ 0.0060 & 1.1587 \\ -0.0377 & 0.3183 \\ 0.0318 & -2.0563 \\ 0.0718 & 1.2516 \\ 0.0752 & -0.1657 \\ -0.1604 & -1.0584 \\ 0.0130 & 2.3314 \\ 0.0410 & -0.4851 \\ -0.0386 & -0.4137 \\ -0.0003 & -0.0017 \\ 0.0455 & 0.0354 \\ -0.0320 & 1.2797 \\ -0.0207 & -0.3598 \\ 0.0190 & -0.2021 \\ -0.0662 & -0.2669 \end{pmatrix} X \begin{pmatrix} A1 \\ A2 \\ A3 \\ A4 \\ A5 \\ A6 \\ A7 \\ A8 \\ A9 \\ A10 \\ A11 \\ A12 \\ A13 \\ A14 \\ A15 \\ A16 \\ A17 \\ A18 \\ A19 \\ A20 \\ A21 \end{pmatrix}$$

The absorbance values of the samples, at the 21 wavelengths in the spectral region from 240 to 340 nm were placed in the above equation and the amounts of Rivaroxaban & Aspirinin the synthetic mixture were found as shown in table 3.

Equation for CLS (Classical Least Square) Method:

The mathematical expression **A=K × C** in the matrix is given as

$$A_1 = K_{11}A_1 + K_{12}C_2 + \ldots\ldots K_{1c}C_c$$

$$A_2 = K_{21}A_1 + K_{22}C_2 + \ldots\ldots K_{2c}C_c$$

$$A_3 = K_{31}A_1 + K_{32}C_2 + \ldots\ldots K_{3c}C_c$$

$$\vdots \quad \vdots \quad \vdots \quad \vdots$$

$$\vdots \quad \vdots \quad \vdots \quad \vdots$$

$$A_w = K_{w1}A_1 + K_{w2}C_2 + \ldots\ldots K_{cw}C_c$$

Where,

A_w = Absorbance at the w^{th} wavelength.

K_{cw} = Calibration coefficient for the c^{th} component at the w^{th} wavelength.

C_c = Concentration of the c^{th} component.

In this method, the calibration coefficient (K) was obtained from the linear equation system using the absorbance data & the training set.

K=pinv(c)*A

The absorbance values of the samples at 21 wavelengths were placed in the above equation & the amounts of Rivaroxaban & Aspirin. in the synthetic mixtures were found, as shown in (Table 7).

Introducing (K) into the linear equation system with an absorbance matrix of sample gives the concentration of Rivaroxaban & Aspirinin the sample mixture.

A1		7.5008	13.8774			
A2		3.6244	6.6989			
A3		1.6501	3.0406			
A4		1.1026	2.0263			
A5		0.9203	1.6890		C_{RIVA}	
A6	=	0.8885	1.6316	X		
A7		1.0156	1.8692		C_{ASP}	
A8		-0.5202	10.1349			
A9		1.7515	3.2390			
A10		2.3041	4.2679			
A11		2.9316	5.4343			
A12		3.5207	6.5289			
A13		3.9203	7.2694			
A14		4.2852	7.9066			
A15		3.8737	7.1787			
A16		3.1434	5.8229			
A17		1.1896	2.2840			
A18		1.1916	2.2120			
A19		0.6312	1.1744			
A20		0.3826	0.7151			
A21		0.2886	0.5403			

The absorbance values of the samples, at the 21 wavelengths in the spectral region from 240 to 340 nm were placed in the above equation and the amounts of Rivaroxaban & Aspirinin the synthetic mixture were found as shown in table 3.

Method validation for Inverse least square method. (As per ICH(Q2) R1 Guideline) [12].

1. Accuracy

Table 3: Recovery results obtained for the determination of Rivaroxaban & Aspirin. Indifferent synthetic mixtures by using the ILS technique.

Rivaroxaban			Aspirin		
Added	**Found**	**% Recovery**	**Added**	**Found**	**% Recovery**
10	9	90	100	106	106
15	14.066	93.77	100	99.98	99.98
20	19.98	99.9	100	99.92	99.92
25	25.31	101.24	100	99.59	99.59
30	29.8211	99.40	100	99.99	99.99
10	10.20	102	150	148.99	99.33
10	10.54	105.4	200	199.89	99.945
10	10.12	101.2	250	248	99.2
10	10.1	101	300	299.65	99.88
10	9.78	97.8	350	350.16	100.39
Mean Recovery		99.171	Mean Recovery		100.47
%RSD		0.27	%RSD		0.6867

2. Precision

Table 4: Data for precision study using one way ANOVA.

Parameters	**ILS**	
	Rivaroxaban	**Aspirin**
Between days variance	0.0479	1.044515
Within days variance	59.0926	9393.971
F ratio	0.000811	0.000111
TSS	1418.318	225457.4

Note that Between day and within day degrees of freedom are 2 and 27, respectively. The critical F ratio value for 2 and 27 d.f and the confidence level of 95% is 3.354

3. Limit of detection (LOD) and Limit of quantification (LOQ)

Table 5: LOD and LOQ values of Rivaroxaban & Aspirinfor ILS method: -

	Rivaroxaban	Aspirin
±SD	0.0081	0.0054
LOD (µg/ml)	0.024	0.016
LOQ (µg/ml)	0.0801	0.054

4. Predicted versus known concentration plot.

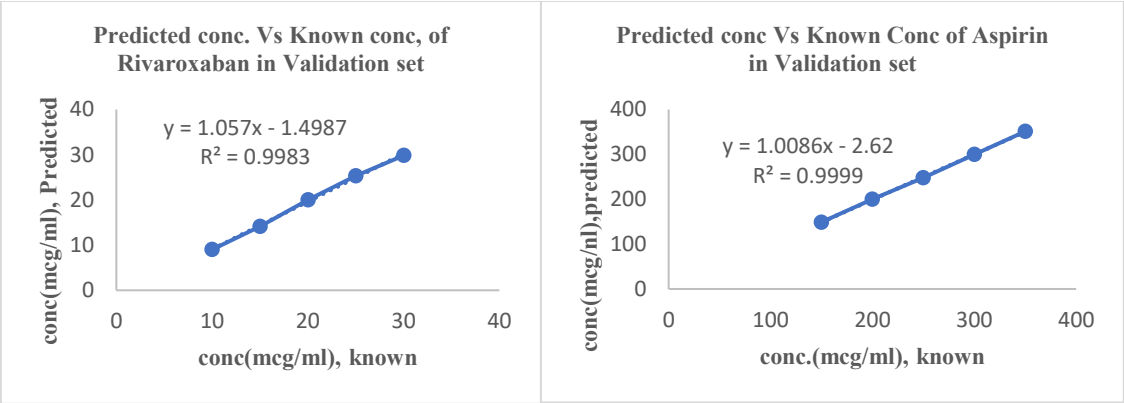


Fig 2: Linearity plots of Rivaroxaban & Aspirinfor validation set of ILS method

It was noticed that both Rivaroxaban & Aspirinin all samples lay on a straight line and the equations of these lines are shown on the graphs (fig. 2).

This indicates that the prediction ability of the validation set is very much better in terms of recovery(Table:3)

5. Concentration residuals versus actual concentration plot.

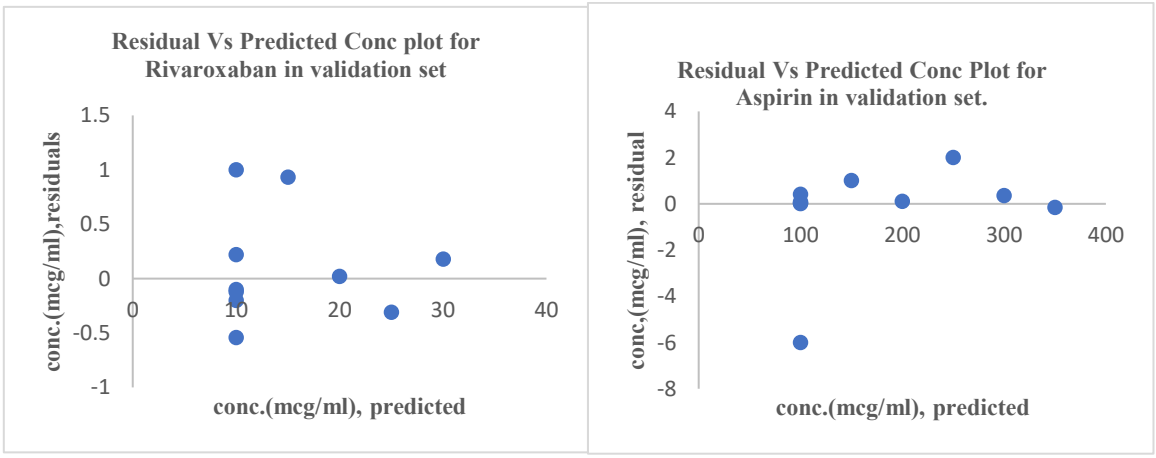


Fig 3: Residual vs. predicted concentration plot for Rivaroxaban & Aspirin.

This tool is used to determine whether the model accounts for the concentration variation in the validation set and it also provides information about how well the method will predict the future samples.

For the validation set it can be found that the residual values are more close to zero and are more randomly distributed. (Fig 3)

6. Root Mean Square Error of Prediction

RMSEP is used for examining the errors in the predicted concentrations.

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{added} - C_i^{found})^2}{n}}$$

It is calculated from the following formula.

Where,

C_i^{added} = added concentration of the drug.

Ci^{found} = predicted concentration of the drug.

n = total number of synthetic mixtures.

The full range of concentration residual should correspond to approximately 2-3 RMSEP units if there is no bias

Table 6: RMSEP values for Rivaroxaban & Aspirinin ILS method.

COMPONENT	RMSEP
RIVA	0.1825
ASP	0.118

Method validation for classical least square method. (As per ICH(Q2) R1 Guideline)[12].

1. Accuracy

Table 7: Recovery results obtained for the determination of Rivaroxaban & Aspirin. In different synthetic mixtures by using the CLS technique.

Rivaroxaban			Aspirin		
Added	**Found**	**% Recovery**	**Added**	**Found**	**% Recovery**
10	10.10	101	100	99.98	99.98
15	14.98	99.86	100	98.56	98.56
20	20.5874	102.93	100	100	100
25	25.77	103.08	100	100.2	100.2
30	30.23	100.76	100	103.5	103.5
10	11.52	115.2	150	150.55	100.36
10	9.8	98	200	198.65	99.325
10	10.2	102	250	256	102.4
10	8.9	89	300	298.63	99.54
10	11.2	112	350	349.56	99.874
Mean Recovery		102.44	Mean Recovery		100.3739
%RSD		0.21	%RSD		0.2154

2. Precision

Table 8: Data for precision studies for Rivaroxaban & Aspirinby one way ANOVA

Parameters	**CLS**	
	Rivaroxaban	**Aspirin**
Between days variance	0.407176	2.7726
Within days variance	59.03818	9378.924
F ratio	0.006897	0.000296
TSS	1417.916	225099.7

Note:- Between day and within day degrees of freedom are 2 and 27 respectively. The critical F ratio value for 2 and 27 df and the confidence level of 95% is 3.354

3. Limit of detection (LOD) and Limit of quantification (LOQ)

Table 9: LOD and LOQ for Rivaroxaban & Aspirinfor CLS method

	Rivaroxaban	Aspirin
*SD	0.0081	0.0054
LOD (µg/ml)	0.024	0.016
LOQ (µg/ml)	0.0801	0.054

4. Predicted versus known concentration plot.

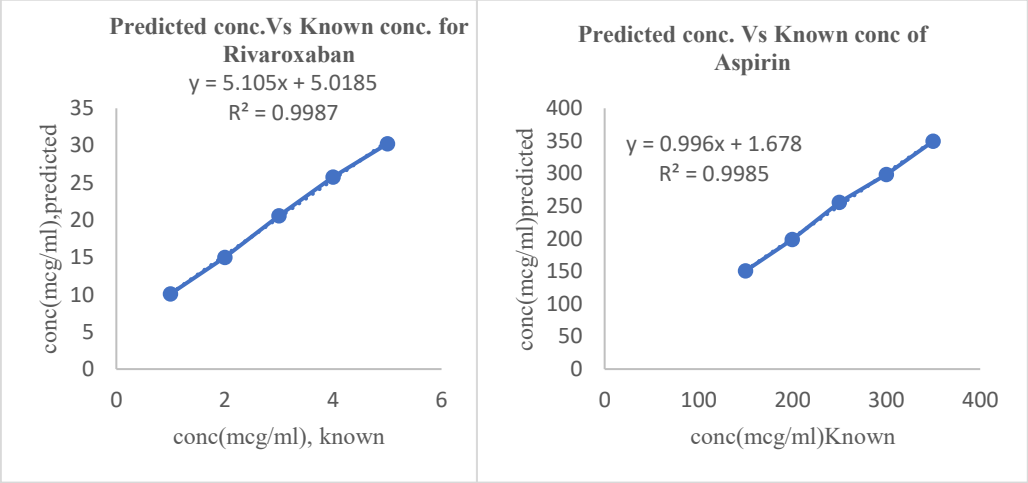


Fig. 4: Linearity plots for Rivaroxaban & Aspirinin CLS method

It was noticed that both Rivaroxaban & Aspirinin all samples lay on a straight line and the equations of these lines are shown on the graphs (Fig. 4).

This indicates that the prediction ability of the validation set is very much better in terms of recovery. (Table 7)

5. Concentration residuals versus actual concentration plot

This tool is used to determine whether the model accounts for the concentration variation in the validation set and it also provides information about how well the method will predict the future samples.

For the validation set it can be found that the residual values are more close to zero and are more randomly distributed[54-61]. (Fig. 5)

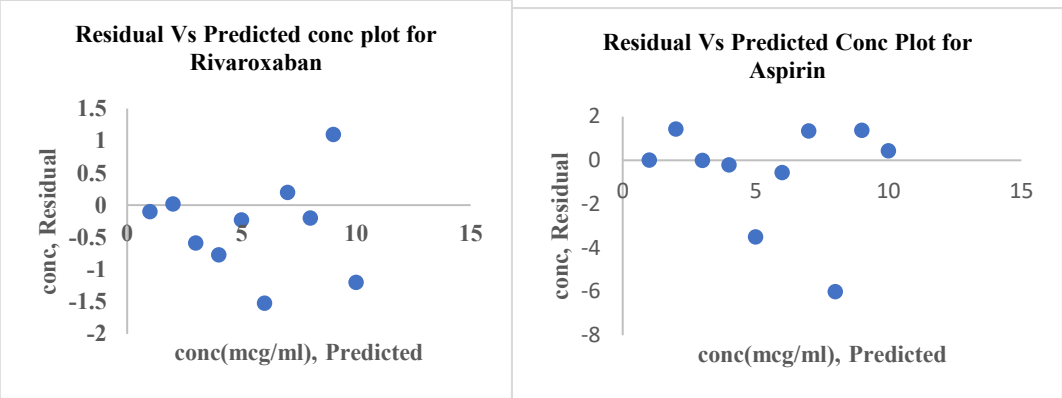


Fig 5: Residual vs. predicted concentration plot for Rivaroxaban&Aspirin

6. Root Mean Square Error Of Prediction

RMSEP is used for examining the errors in the predicted concentrations.

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{added} - C_i^{found})^2}{n}}$$

It is calculated from the following formula.

Where,

C_i^{added} = added concentration of the drug.

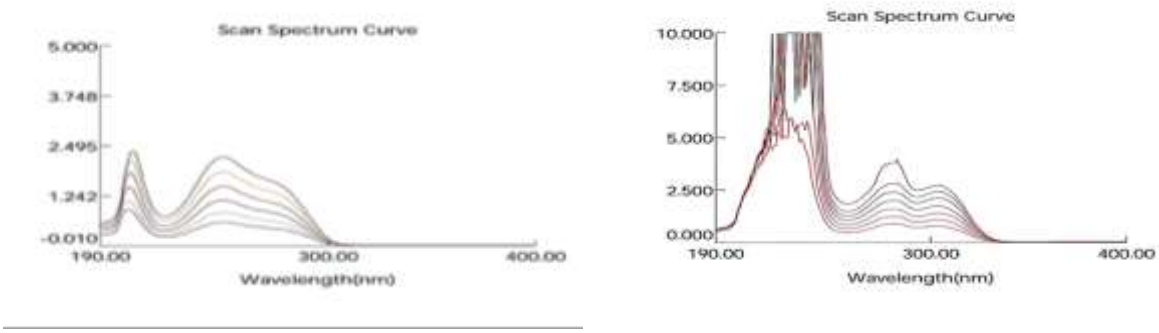
Ci^{found} = predicted concentration of the drug.

n = total number of synthetic mixtures.

The full range of concentration residual should correspond to approximately 2-3 RMSEP units if there is no bias.

Table 10: RMSEP values for Rivaroxaban and Aspirin for CLS method

COMPONENT	RMSEP
Rivaroxaban	0.3687
Aspirin	0.5



(a) (b)

Fig 6: Overlay spectrum of (a)Rivaroxaban and (b)Aspirin showing various spectra at different concentration of drug

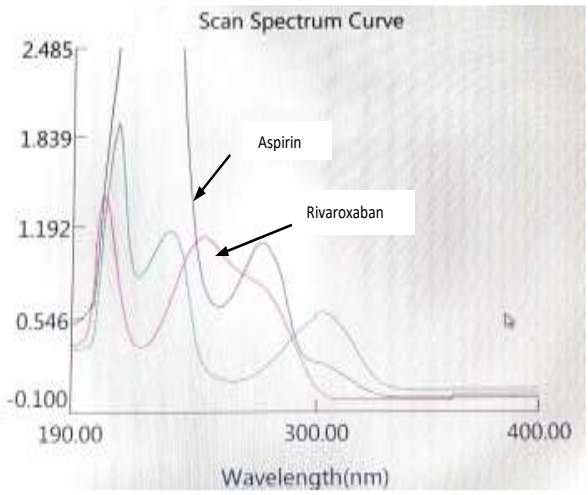
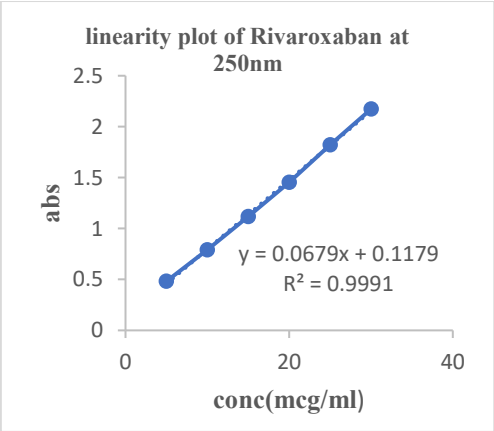


Fig 7: Overlay spectra of Rivaroxaban and Aspirin [11]



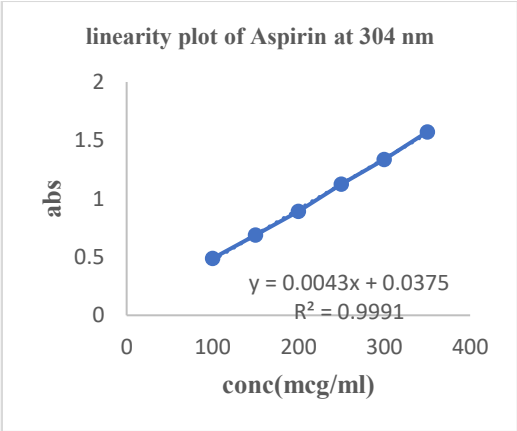


Fig 8: Calibration Plots of Rivaroxaban **Fig 9: Calibration Plots of Aspirin**

Statistical analysis

Table 11: - Statistical comparison of the results obtained from Simultaneous equation method & Q Analysis

Drug (1:10)	**Simultaneous equation method**		**Q analysis**	
	Rivaroxaban	**Aspirin**	**Rivaroxaban**	**Aspirin**
Labelled claim	15mg	150mg	15mg	150mg
Mean ± SD	14.78mg ± 1.7	152.84mg ± 1.48	14.98mg ±1.70	150.41mg ± 1.48
No of samples	5	5	5	5
t theoretical	5.768		**F therotical**	6.388
t calculated			**F calculated**	
For Rivaroxaban	0.3141		For Rivaroxaban	0.6171
For Aspirin	0.187		For Aspirin	0.769697

Table 12: Results of ANOVA test for Rivaroxaban & Aspirin obtained in Synthetic mixture by using simultaneous equation method, Q-analysis &chemometric method of analysis (ILS & CLS)

Source of Variation	**Sum of square**		**Degree of freedom**		**Mean sum of square**		**F test***	
	Rivaroxaban	**Aspirin**	**Rivaroxaban**	**Aspirin**	**Rivaroxaban**	**Aspirin**	**Rivaroxaban**	**Aspirin**
Between Groups	3.795	1195.63	3	3	1.26	398.54	0.021	0.117
Within Groups	952.08	4.786.8	16	12	59.506	3398.9		

* F theoretical =3.238 at P=0.9

Conclusion:

Comparing to other conventional methods, chemometric techniques do not imply any pretreatment such as the separation procedure in HPLC, This methods were developed for Rivaroxaban and Aspirin incombinations. In addition, ILS & CLS has been found to be the

method of choice for more complex mixtures (ternary or quaternary). Chemometric techniques are very easy to apply, they require only data processing with powerful software and its application to the regression analysis.

Thus, the developed methods enable the quantitation of mixture with good accuracy & precision, either in laboratory-prepared samples or in commercial pharmaceutical dosage forms.

Acknowledgements

The authors like to extend heartfelt thanks to Megafine, Mumbai and Wockhardt research Centre Aurangabad for providing gift sample of Rivaroxaban and Aspirin resp. The authors also acknowledge P. R. Pote Patil College of Pharmacy, Amravati for providing Facilities to conduct this research.

Conflict of interest

I/We certify that no actual or potential conflict of interest in relation to this article exists.

References

1. Rathod S.M, Patel P.U. Chemometrics- assisted spectrophotometric Method Development and validation for simultaneous estimation of emtricitabine, Tenofovir Alafenamide Fumarate, and Dolutegravir Sodium in Dosage form. Published by Wolters Kluwer-Medknow, *jrpsjournal*, (2023) 41-50.
2. Zhdan H, Sarkis N, Dual Wavelength and first order Derivative spectrophotometric Methods for Simultaneous Estimation of Rivaroxaban and Aspirin in Synthetic Mixture. International Research Journal of Pure & Applied Chemistry 21(24)(2020):48-54
3. KD Tripathi, Essential of Medical Pharmacology published by Jaypee brothers medical publisher , 8th edition (2019)
4. Alban S: Pharmacological strategies for inhibition of thrombin activity. Curr Pharm Des. 2008;14(12):1152-75
5. FDA Approved Products: XARELTO (rivaroxaban) tablets or suspension, for oral use 0224
6. E. Dinc¸ A, D. Baleanu B. C, Il Farmaco, Two new spectrophotometric approaches to the multicomponent analysis of the acetaminophen and caffeine in tablets by classicalleast-squares and principal component regression techniques, (2002) (57) 33–37.
7. Ghasemi J, Abbasi B, Niazi A, Simultaneous spectrophotometric multicomponent determination of folicacid, thiamine, riboflavin, and pyridoxalby using double divisor–

ratio spectra derivative–zero crossing method. Analytical Letters, (2004) Vol. 37, No. 12, Pp. 2609–2623.

8. Salem M.Y, Mohamed G. El-Bardicy, Simultaneous determination of domperidone maleate & cinnarizine in a binary mixture using derivative ratio spectrophotometry & classical least squares calibration. *Journal Of Pharmaceutical & Biomedical Analysis*. (2002) (33)21-33.
9. Simultaneous determination of methylxanthines in coffees& teas by uv-vis. & PLS, *Analytica Chimica Acta* 493(2003),83-94
10. Kamal A.H, et.al, A Review on UV Spectrophotometric methods for simultaneous multicomponent analysis. European journal of pharmaceutical and medical research (2016),3(2) 348-360.
11. Beckett AH. And Stennlake JB. Practical pharmaceutical chemistry: Part-2 :4th edition; CBS Publishers and distributors, New Delhi, 2002, pp 264-275,275-300.
12. Validation of Analytical Procedures: Text and Methodology Q2(R1), ICH Harmonised Tripartite guidelines, International Conference on Harmonisation of Technical Requirements For Registration Of Pharmaceuticals For Human Use, 2005; 13
13. Sarkis N, Bitar Y, Sarraj M.M, Development, and validation of RP-HPLC Method for simultaneous estimation of Aspirin and Rivaroxaban in synthetic Mixture. Research J. Pharm. And Tech.13(11) (2020): 5459-5465
14. U.S. FDA Approves XARELTO® (rivaroxaban) to Reduce the Risk of Major Cardiovascular Events in Patients with Chronic Coronary Artery Disease (CAD) or Peripheral Artery Disease (PAD) [news release]. Titusville, NJ; October 11, 2018: Janssen Pharmaceuticals website. https://www.janssen.com/us-fda-approves-xareltor-rivaroxaban-reduce-risk-major-cardiovascular- events-patients-chronic. Accessed October 12, 2018
15. IP 2010, Government of India, Ministry of health and family welfare, Ghaziyabad, pp1657-1658
16. Kotecha N, Development and validation of analytical methods for the estimation of anti-diabetic drugs published by gtusitecirculars phd thesis 2019 ; page no 1-231
17. Mousumi K, Choudhury PK. HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. Indian J Pharm Sci. 2009;71(3):318-20.

18. Vasudevan M, Ravi J, Ravisankar S, Suresh B. ION-pair liquid chromatography technique for the estimation of metformin in its multicomponent dosage forms. J Pharmaceut Biomed. 2001;25(1):77-84.
19. Gandhimathi M, Anandakumar K, Cheriyan A, Ravi T. Simultaneous estimation of metformin and gliclazide in tablets using reverse phase high performance liquid chromatography. Indian J Pharm Sci. 2003;65(5):530-531.
20. Lad NR, Bhoir SI, BhoirI C, Sundaresan M. Concurrent assay of metformin and glimepiride in tablets using RP-HPLC with wavelength programming. Indian J Pharm Sci. 2003;65(6):650- 3
21. Fang J, Alderman M.H. Dissociation of hospitalization and mortality trends for myocardial infarction in the United States from 1988 to 1997. Am. J. Med. 2002;113:208–214. doi: 10.1016/S0002-9343(02)01172-5. [PubMed] [CrossRef] [Google Scholar]
22. Feigin V.L, Lawes C.M, Bennett D.A, Anderson C.S. Stroke epidemiology: A review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. Lancet Neurol. 2003;2:43–53. doi: 10.1016/S1474-4422(03)00266-7. [PubMed] [CrossRef] [Google Scholar]
23. White R.H. The Epidemiology of Venous Thromboembolism. Circulation. 2003;107:I-4–I-8. doi: 10.1161/01.CIR.0000078468.11849.66. [PubMed] [CrossRef] [Google Scholar]
24. Mackman N., Bergmeier W., Stouffer G.A., Weitz J.I. Therapeutic strategies for thrombosis: New targets and approaches. Nat. Rev. Drug Discov. 2020;19:333–352. doi: 10.1038/s41573-020-0061-0. [PubMed] [CrossRef] [Google Scholar]
25. Ten Cate H, Hackeng T.M, de Frutos P.G. Coagulation factor and protease pathways in thrombosis and cardiovascular disease. Thromb. Haemost. 2017; 117:1265–1271. doi: 10.1160/TH17-02-0079. [PubMed] [CrossRef] [Google Scholar]
26. Heestermans M, Poenou G, Hamzeh-Cognasse H, Cognasse F, Bertoletti L. Anticoagulants: A Short History, Their Mechanism of Action, Pharmacology, and Indications. Cells. 2022 Oct 13;11(20):3214. doi: 10.3390/cells11203214. PMID: 36291080; PMCID: PMC9600347.
27. Katzung G.K, basic and clinical pharmacology published by Mc Graw Hill Education 14th edition (2018) page no 608-610
28. https://www.nagwa.com/en/explainers/323174105238/

29. Melillo SN, Scanlon JV, Exter BP, Steinberg M, Jarvis CI: Rivaroxaban for thromboprophylaxis in patients undergoing major orthopedic surgery. Ann Pharmacother. 2010 Jun;44(6):1061-71. doi: 10.1345/aph.1M681. Epub 2010 Apr 27.
30. Ufer M: Comparative efficacy and safety of the novel oral anticoagulant's dabigatran, rivaroxaban and apixaban in preclinical and clinical development. Thromb Haemost. 2010 Mar;103(3):572-85. doi: 10.1160/TH09-09-0659. Epub 2010 Feb 2.
31. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE: The Protein Data Bank. Nucleic Acids Res. 2000 Jan 1;28(1):235-42.
32. Jonathan P Piccini , Manesh R Patel , Kenneth W Mahaffey , Keith AA Fox & Robert M Califf (2008) Rivaroxaban, an oral direct factor Xa inhibitor, Expert Opinion on Investigational Drugs, 17:6, 925-937, DOI: 10.1517/13543784.17.6.925
33. Alfonso L, Ai G, Spitale RC, Bhat GJ: Molecular targets of aspirin and cancer prevention. Br J Cancer. 2014 Jul 8;111(1):61-7. doi: 10.1038/bjc.2014.271. Epub 2014 May 29
34. Vane JR, Botting RM: The mechanism of action of aspirin. Thromb Res. 2003 Jun 15;110(5-6):255-8
35. Ornelas A, Zacharias-Millward N, Menter DG, Davis JS, Lichtenberger L, Hawke D, Hawk E, Vilar E, Bhattacharya P, Millward S: Beyond COX-1: the effects of aspirin on platelet biology and potential mechanisms of chemoprevention. Cancer Metastasis Rev. 2017 Jun;36(2):289-303. doi: 10.1007/s10555-017-9675-z.
36. Mayo Clinic website: Salicylate
37. Durlaza FDA label
38. Flower R: What are all the things that aspirin does? BMJ. 2003 Sep 13;327(7415):572-3. doi: 10.1136/bmj.327.7415.572. [Article]
39. Hall MN, Campos H, Li H, Sesso HD, Stampfer MJ, Willett WC, Ma J: Blood levels of long-chain polyunsaturated fatty acids, aspirin, and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2007 Feb;16(2):314-21. [Article]
40. Vane JR, Botting RM: The mechanism of action of aspirin. Thromb Res. 2003 Jun 15;110(5-6):255-8.
41. Li D, Wang P, Yu Y, Huang B, Zhang X, Xu C, Zhao X, Yin Z, He Z, Jin M, Liu C: Tumor-preventing activity of aspirin in multiple cancers based on bioinformatic analyses. PeerJ. 2018 Sep 26;6:e5667. doi: 10.7717/peerj.5667. eCollection 2018

42. Tsoi KKF, Ho JMW, Chan FCH, Sung JJY: Long-term use of low-dose aspirin for cancer prevention: A 10-year population cohort study in Hong Kong. Int J Cancer. 2018 Dec 21. doi: 10.1002/ijc.32083
43. Vane JR, Bakhle YS, Botting RM: Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol. 1998;38:97-120. doi: 10.1146/annurev.pharmtox.38.1.97
44. Levy G: Clinical pharmacokinetics of aspirin. Pediatrics. 1978 Nov;62(5 Pt 2 Suppl):867-72.
45. Authors unspecified: Guidance document: management priorities in salicylate toxicity. J Med Toxicol. 2015 Mar;11(1):149-52. doi: 10.1007/s13181-013-0362-3.
46. Durnas C, Cusack BJ: Salicylate intoxication in the elderly. Recognition and recommendations on how to prevent it. Drugs Aging. 1992 Jan-Feb;2(1):20-34.
47. KD Tripathi, Essential of Medical Pharmacology published by Jaypee brothers medical publisher , 8th edition (2019) page no 667-668
48. Aldabib J. And Edbeib M., 2020. The effects of concentration based on the absorbance form the ultraviolet–visible (UV-VIS) spectroscopy analysis. International Journal of Science Letters, 2(1), pp.1-11.
49. Verma G. and Mishra M., 2018. Development and optimization of UV-Vis spectroscopy-a review. World J. Pharm. Res, 7(11), pp.1170-1180.
50. Shinde G., Godage R.K., Jadhav R.S., Manoj B. and Aniket B., 2020. A Review on Advances in UV Spectroscopy. Research Journal of Science and Technology, 12(1), pp.47-51.
51. Malarat, N, Oin, W, Kanjana, K, Makkliang, F, Siaj, M. and Poorahong, S, 2023. Electrochemical platform based on activated carbon/graphene oxide-gold nanoparticle composites for the electrochemical sensing of methylparaben in cosmetic samples. Microchemical Journal, 188, p.108473.
52. Sharma S, Goyal S, Chauhan K. A Review on analytical method development and validation. International journal of applied pharmaceutics. (2018) vol. 10, issue 6,8-15.
53. Simultaneous estimation of chloroxazone & tramadol hcl. In tablet formulation by two wavelength spectrophotometry. *The Pharma Review*, (2005), Volume 4, No.19
54. Salpe, H. G., Devhare, L. D., Ghugare, A. P., & Singh, N. (2016). Formulation and evaluation of hpmc coated diltiazem hcl tablet and its comparison with other marketed preparation. Research chronicle in health sciences, 3(1), 11-17.
55. Devhare, L., Hiradeve, S., & Bobade, T. (2017). Method Development & Validation For Determination Of Water Content. LAP LAMBERT Academic Publishing.

56. Devhare, L. D., Ghugare, A. P., Hatwar, B. P., Goupale, D. C., & Devhare, L. D. (2015). Method development for determination of water content from various materials by spectrophotometry and it's validation. International journal of drug delivery, 7(4), 233-240.
57. Tonde, T. U., Kasliwal, R. H., & Devhare, L. D. (2016). Quantitative estimation of bacoside a in polyherbal memory enhancer syrup for memory boosting activity using hptlc method. Research chronicle in health sciences, 2(6), 315-320.
58. Ghugare, A. P., Devhare, L., & Hatwar, B. P. (2016). Development and validation of analytical methods for the simultaneous estimation of Nimorazole and Ofloxacin in tablet dosage form. International Journal of Drug Delivery, 8, 96-98
59. Makhani, A. A., & Devhare, L. (2017). Development and validation of vierordt's spectrophotometric method for simultaneous estimation of Drotaverine and Nimesulide combination. Research chronicle in health sciences, 3(2), 22-28
60. Makhani, A. A., & Devhare, L. D. (2017). Development and validation of analytical methods for drotaverine and nimesulide combination. Research chronicle in health sciences, 3(3), 40-44.
61. Katole, G., & Devhare, L. (2018). Diluent and granulation study on Metformin Hydrochoride. LAP LAMBERT Academic Publishing.