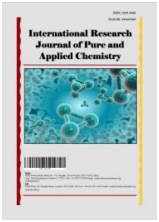


International Research Journal of Pure & Applied Chemistry

21(24): 48-54, 2020; Article no.IRJPAC.63616
ISSN: 2231-3443, NLM ID: 101647669

Dual Wavelength and First Order Derivative Spectrophotometric Methods for Simultaneous Estimation of Rivaroxaban and Aspirin in Synthetic Mixture

Heba Zhdan[1] and Nazira Sarkis[1*]

[1]Department of Analytical and Food Chemistry, Faculty of Pharmacy, University of Aleppo, Syria.

Authors' contributions

This work was carried out in collaboration between both authors. Author HZ performed the experiments, put the characterization protocols and wrote the first draft of the manuscript. Author NS managed the project in all stages, contribute to the interpretation of data and revised the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2020/v21i2430334
Editor(s):
(1) Dr. Richard Sawadogo, Research institute for health sciences, Burkina Faso.
Reviewers:
(1) Adegbite Adesola, Ladoke Akintola University of Technology, Nigeria.
(2) Ahmed M. Sadoon, University of Mosul, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63616

Original Research Article

Received 14 October 2020
Accepted 19 December 2020
Published 31 December 2020

ABSTRACT

Two simple, sensitive, accurate and precise spectrophotometric methods were developed and validated for simultaneous estimation of binary mixture of Rivaroxaban and Aspirin in their pure form and synthetic mixture in difficult ratio *1:20(*Rivaroxaban: Aspirin). The first method is the dual wavelength method, where *250* nm and *286.44* nm were selected as λ_1 and λ_2 for determination. And the two wave length for determination of Aspirin were *243.53* nm and *259.2* nm. The linearity range was studied over concentration ranges (2 – 12) µg/mL and (40-240) µg/mL for Rivaroxaban and Aspirin respectively in both methods, with correlation coefficients not less than 0.9996 and up to 0.9999 respectively in a row. The limit of detection (LOD) and limit of quantification (LOQ) were 0.24µg/mL and 0. 82µg/mL for Rivaroxaban and for Aspirin *were7.03* µg/mL and 22.01 µg/mL respectively in the first method. The second method is First order derivative. The wavelengths 275.25 nm and 250 nm were selected as zero crossing point to determine Rivaroxaban and Aspirin in a row. The LOD and LOQ were 0.31 µg/mL and 0.95 µg/mL for Rivaroxaban; and for Aspirin was found to be*7.13* µg/Ml and *21.60*µg/mL respectively. The two methods were successfully validated as per ICH guidelines. Both methods were simple, sensitive and rapid.

**Corresponding author: E-mail: nazirasarkis@gmail.com; peanut.heb@gmail.com;*

Keywords: Rivaroxaban; aspirin; dual wave length; first order derivative; synthetic mixture.

1. INTRODUCTION

Rivaroxaban is 5-chloro-*N*-[[(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide). has molecular weight, 435.881 g/mol), (Fig. 1A). Rivaroxaban is classified as an oral anticoagulant. Its mechanism is an antithrombin independent, where it's an active direct Factor Xa inhibitor within the coagulation cascade. Rivaroxaban is licensed in the EU for Prevention of venous thromboembolism, Prevention of stroke when patients suffer from atrial fibrillation and atherothrombotic events, and for Treatment of deep-vein thrombosis [1].

Aspirin is 2- (acetyloxy) benzoic acid. It is classified as cyclo oxygenase inhibitor and best known as an antiplatelet drug, Aspirin is a weak acid, it is white crystalline powder, and has molecular mass – 180.157 g/mol, (Fig. 1B). One of the major antithrombogenic agent is Aspirin. It's widely used for the prevention and treatment of cardiovascular and cerebro strokes, Aspirin reduces risks of cardiovascular conditions after coronary artery bypass graft, and acute myocardial infarction [2].

The US Food and Drug Administration (FDA) approved the combination of Rivaroxaban with Aspirin to offer a new option for treatment and prevention of major cardiovascular events in patients with elevated cardiac biomarkers, and for patients with atrial fibrillation ,and chronic coronary artery disease; and for patients after surgeries like transcatheter aortic valve replacement [3-4].

Rivaroxaban revealed several analytical methods based on varied techniques Like (RP-HPLC) [5-9], Liquid Chromatography and Mass Spectroscopy (LC-MS) [10], UV spectrophotometry [11-13] were reported to determine Rivaroxaban as individual or combination with another drug.

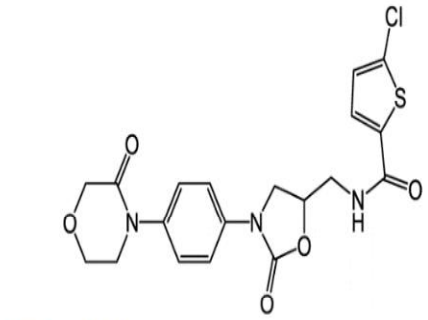
Similarly, a survey of the analytical literature for Aspirin revealed methods based on (RP-HPLC) Reverse Phase High Performance Liquid Chromatography [14-18], (HPTLC) High Performance Thin Liquid Chromatography [19], (GC-MS) Gas Chromatography and Mass Spectroscopy [20], UV spectrophotometry for determination in pharmaceuticals [21-24], and spectrophotometric method for determination of Aspirin in blood samples [25].

No spectrophotometric method was found to determine this combination. So, the aim of this work was to develop and validate a simple, rapid and highly sensitive spectrophotometric method for the determination of Aspirin and Rivaroxaban in synthetic mixture.

2. MATERIALS AND METHODS

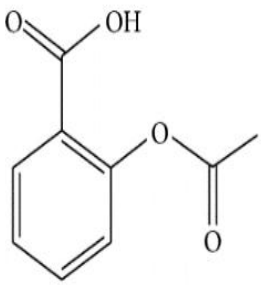
2.1 Instruments

The UV Spectrophotometric instrument is a (T80+ UV/V spectrophotometer instrument Ltd (UK)) connected to computer. With 1 cm Quartz cells. Analytical balance (Sartorius, model 2474, Germany). Adjustable micro pipettes 100 to 1000 µL (LABGILLS, Germany). Ultrasonic bath (Power sonic, model 405, Korea).



RIVAROXABAN

(A)



ASPIRIN

(B)

Fig. 1. The chemical structures of : A- rivaroxaban, B- aspirin

2.2 Reagents and Chemicals

Standard active pharmaceutical ingredients of Aspirin (99.7%) and Rivaroxaban (99.9%).
Acetonitrile (Honeywell, France, HPLC grade).

2.3 Preparation of Standard Solutions

2.3.1 Stock solution of rivaroxaban

After weighing equivalent to 25 mg of Rivaroxaban. It was transferred into 25 ml volumetric flask and diluted with acetonitrile to get standard stock solution 1000 µg/ml of Rivaroxaban. Then from above 2.5 ml was pipetted out in 25 ml volumetric flask and made up to the mark with acetonitrile to make final concentration of Rivaroxaban 100 µg/ml.

2.3.2 Stock solution of aspirin

After weighing equivalent to 25 mg of Aspirin. It was transferred into 25 ml volumetric flask and diluted with acetonitrile to get standard stock solution 1000 µg/ml of Aspirin.

2.3.3 Preparation of synthetic mixture of rivaroxaban and aspirin

The synthetic mixture of Rivaroxaban(RXN) and Aspirin(ASP) was made in the ratio 1:20 (Rivaroxaban:Aspirin). After weighing equivalent to 2.5 mg of Rivaroxaban and 50 mg of Aspirin. Powder was transferred and added to other excipients 88 mg dilute Lactose Monohydrate, 15 mg Micro Crystalline Cellulose MCC, 4 mg Povidone, 1mg Talc, and 2 mg Magnesium stearate into Petri dish. Then, taken into 25 ml volumetric flask. Added 15 ml of Acetonitrile and sonicated it. Diluted up to the mark with Acetonitrile. This solution was filtered through filter paper. The mixture contains 100 µg/ml Rivaroxaban and 2000 µg/ml Aspirin. Then 250 µl from the last solution was pipetted out in 5 ml volumetric flask and made up to the mark with acetonitrile to make final solution in which concentration of Rivaroxaban is 5 µg/ml and concentration of Aspirin is 100 µg/ml in respect of the ratio 1:20.

3. RESULTS AND DISCUSSION

3.1 Dual Wave Length Spectrophotometric Method

This method is depending on the zero-order absorption spectra and it is time and effort saving and gives excellent accuracy .It solves the overlapping problem without any changes in the zero-order absorption spectrum of the combination [26-28].

For the determination of Rivaroxaban; two wavelengths (250 nm and 286.44 nm) were selected where the absorbance difference between the two wavelengths is directly proportional to the concentration of Rivaroxaban and the absorbance difference of Aspirin at these wavelengths is zero.

While for the determination of Aspirin; two wavelengths (243.53 nm and 259.20 nm) were selected where the absorbance difference between the two wavelengths is directly proportional to the concentration of Aspirin and the absorbance difference of Rivaroxaban at these wavelengths is zero.

3.2 First Order Derivative Spectrophotometric Method

First derivative spectrophotometric method depends on zero crossing point. This method is depending on the first-order absorption spectra and it is easy, fast and gives maximum accuracy. It solves the overlapping problem.[26] We could determine Rivaroxaban at 275.25 nm and Aspirin at 250 nm according to this method.

3.3 Linearity

For the two methods six concentrations of Rivaroxaban from 2 µg/ml to 12 µg/ml were taken, and six concentrations of Aspirin from 40 µg/ml to 240 µg/ml. Each concentration was repeated six duplicates. Results are shown in Table 1.

3.4 Accuracy

We prepared three different concentrations of each drug, and each concentration was repeated three times, to determine the accuracy of the two methods. Results are shown in Table 1.

Accuracy of the methods was more confident by the use of the standard addition

3.5 Precision

Repeatability precision (the intra-day precision): is the mean of three different concentrations & each concentration was repeated three times in day.

Intermediate precision (the inter-day precision): is the average of three different concentrations & each concentration repeated three times in two successive days.

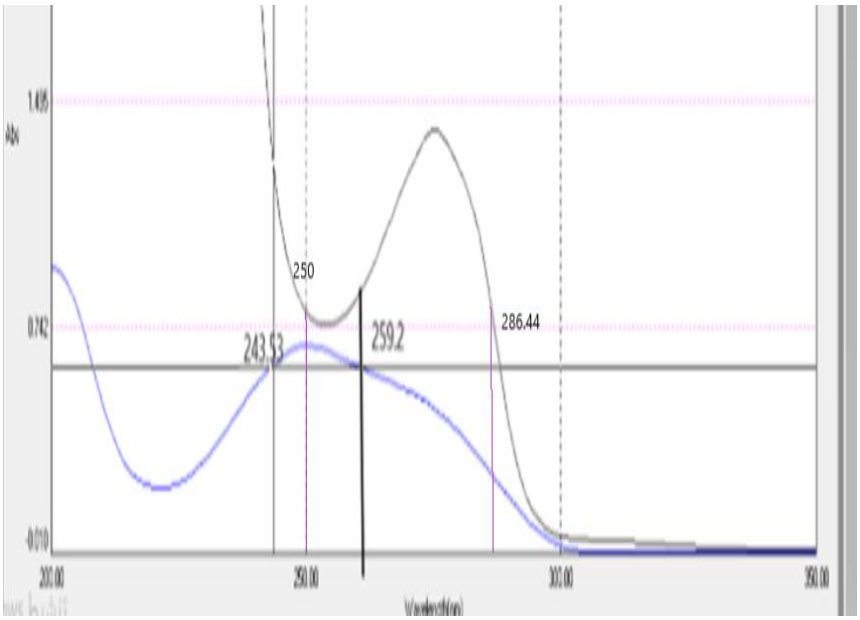


Fig. 2. Dual wave length method to determine rivaroxaban and aspirin

Table 1. Assay parameters and methods validation

Methods	Dual wavelength method		First derivative method	
Parameters	**RXN[1]**	**ASP[2]**	**RXN**	**ASP**
Wave lengths nm	ΔA between 250 , 286.44	ΔA between 243.53 , 259.2	275.25	250
Linearity-range(µg/ml)	2-12	40-240	2-12	40-240
Regression equation	Y=0.0552X-0.0001	Y=0.0022X+0.0452	Y=0.0012-0.0002	Y=0.0001X-0.0003
Correlation coefficient	0.9996	0.9999	0.9997	0.9999
LOD µg/ml	0.24	7.03	0.31	7.13
LOQ µg/ml	0.82	22.01	0.95	21.6
*Accuracy (mean±RSD%)	100.39±0.28	99.72±0.37	99.95±0.94	100.93±1.26

1 abbreviation of Rivaroxaban
2 abbreviation of Aspirin

Table 2. The accuracy data of the developed methods

Amt. of sample (µg/ml)		Amt. of drug added (µg/ml)		% Recovery (n=3) *			
				Dual wavelength		First derivative method	
RXN[1]	**ASP[2]**	**RXN**	**ASP**	**ASP**	**RXN**	**ASP**	**RXN**
5	100	4	80	99.57	100.87	99.40	100.24
5	100	5	100	99.75	99.73	100.13	100.33
5	100	6	120	100.07	100.36	100.23	100.57
Mean ± SD				99.79±0.42	100.32±0.28	99.92±0.63	100.38±0.56

** n = number of repetitions.*
[1] abbreviation of Rivaroxaban
[2] abbreviation of Aspirin

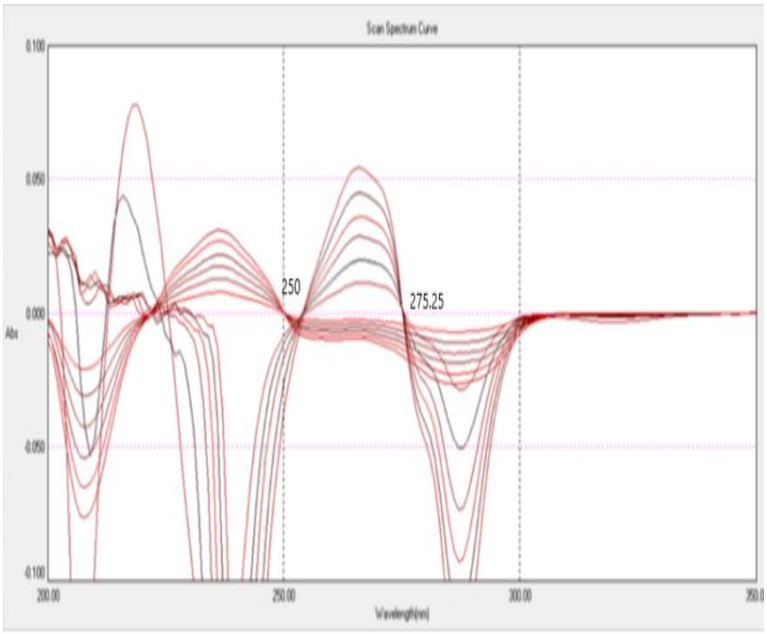


Fig. 3. First derivative method to determine rivaroxaban and aspirin

Table 3. Result of analysis of synthetic mixture

Methods	Dual wavelength method		First derivative method	
	ASP[1]	RXN[2]	ASP	RXN
%Assay	99.66	101.00	99.83	100.73
SD(n*=3)	0.21	0.25	0.20	0.21

n = number of repetitions
[1] abbreviation of Aspirin
[2] abbreviation of Rivaroxaban

Table 4. The precision data of the developed methods (n=3)*

Method		Dual wavelength method (%RSD)		First derivative method (%RSD)	
		RXN	ASP	RXN	ASP
System precision	Intraday	0.98	0.42	1.09	1.39
	Interday	0.95	0.55	1.05	1.35

n = number of repetitions

4. CONCLUSION

Two simple, rapid, accurate and precise UV spectrophotometric methods were developed and validated to estimate Rivaroxaban and Aspirin in synthetic mixture in difficult ratio 1:20 (Rivaroxaban : Aspirin).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, there search was not funded by the producing company rather it was funded by personal efforts.

ACKNOWLEDGEMENTS

The authors acknowledge the technical support received from university of Aleppo, faculty of pharmacy, Aleppo, Syria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. European Medicines Agency (EMEA). CHMP Assessment Report for Xarelto. Accessed; 2010. Available:ttp://www.emea.europa.eu/humandocs/PDFs/EPAR/ xarelto/H-944-en6.pdf. 2008.
2. Hennekens CH, Sechenova O, Hollar D, Serebruary VL. Dose of aspirin in the treatment and prevention of cardiovascular disease: Current and future directions, Journal of Cardiovascular and Pharmacology. 2006;3(11):170–176.
3. Summary LAY. Rivaroxaban (Xarelto) in combination with aspirin for prevention of major cardiovascular events in coronary or peripheral artery disease. National Institute for Health Research. 2017;1–8.
4. FDA Okays Rivaroxaban Plus Aspirin for Chronic CAD. PAD – Medscape. 2018. Available:https://www.medscape.com/viewarticle/903344.
5. Srinivasrao V, Girase YN, Diptisoni. Development and validation of stability indicating RP-HPLC method for rivaroxaban and Its impurities**.** SOJ Biochemistry. 2018;4(1):1-6.
6. Sunny A, Sreedhar C, Akkama HG, Mahapatra A. Development of new analytical method and validation for quantitative estimation of rivaroxaban in formulation and bulk drug. International Journal of Scientific Research in Education. 2017;5(5):6469-6478.
7. Avachat AM, Yamgar MP. RP-HPLC method development and validation for the estimation of rivaroxaban in bulk and tablet dosage form. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;6(8):1775-1784.
8. Shivashankar V, Gandhimathi M, Ravi TK Development of validated RP-HPLC method for estimation of rivaroxaban in pharmaceutical formulation, International Journal of Pharmaceutical Research and Analysis. 2015;4(4):406-410.
9. Sahoo S, Mekap SK. Assay comparison of rivaroxaban by new HPLC method with an existing method in tablet dosage form. Pharmaceutical and Biological Evaluations. 2017;4(3):180-182.
10. Arous B, Al-Mardini MA, Ghazal H, Al-Lahham F. Sability-indicating method for the determination of rivaroxaban and its degradation products using LC-MS, and TLC. Research J. Pharm and Tech. 2018;11(1):3.
11. Seshamamba BSV, Sekaran CB. Spectrophotometric quantification of direct factor xa inhibitor, rivaroxaban, in raw and tablet dosage form. Glob Drugs and Therap. 2017;2(3):1-8.
12. Çelebier M, Kaynak MS, Altinöz S, Sahin S. UV spectrophotometric method for determination of the dissolution profile of rivaroxaban. Dissolution Technologies. 2014;21:56-59.
13. Sekaran CB, Bind VH, Damayanthi MR, Sireesha A. Development and validation of UV spectrophotometric method for the determination of rivaroxaban, Der Pharma Chemica. 2013;5(4):1-5.
14. Kalshetti MS, Kasabe S, Chauhan N, Dhanshri S, Kale B. Development and validation of RP-HPLC method for simultaneous estimation of aspirin and omeprazole in dosage. International Journal of Research in Pharmacy and Pharmaceutical Sciences. 2017;2(4): 45-5.
15. Kiran KK, Supriya D, Divya D, Rani D, Munni GN. Analytical method development and validation for the estimation of aspirin and omeprazole using RP-HPLC method. Intercontinental journal of pharmaceutical Investigations and Research. 2017;4(1):44-71.
16. Mabunni SK, Kumari AS, Kiran PC. A new RP-HPLC method development and validation for simultaneous estimation of aspirin and omeprazole in bulk and pharmaceutical dosage forms. Intercontinental Journal of Pharmaceutical Investigations and Research. 2017; 4(3):240-245.
17. Chodvadiya FJ, Thula KC, Maheshwari DG. Simultaneous estimation of aspirin and lansoprazole by RP–HPLC method, International Journal of Recent Scientific Research. 2015;6(4):3385-3390.
18. Rajput S, Fanse S. RP-HPLC method for simultaneous estimation of lansoprazole and aspirin in bulk and laboratory mixture. Journal of Advanced Pharmacy Education & Research. 2015;5(2):240-245.
19. Kumar S, Jamadar LD, Bhat K, Musmade P, Vasantharaju SG. Analytical method development and validation for aspirin.

International Journal of Chem Tech Research. 2010;2(1):389-399.

20. Tsikasa D, Tewesa KS, Gutzkia FM, Schwedhelma E, Greipel J, Frolich JC. Gas Chromatographic-tandem mass Spectrometric determination of acetylsalicylic acid in human plasma after oral administration of low-dose aspirin. Journal of Chromatography B: Biomedical Sciences and Applications. 1998;709:79-88.
21. Kokot Z, Burda K, Simultaneous determination of salicylic acid and acetylsalicylic acid in aspirin delayed-release tablet formulations by second-derivative UV spectrophotometry. Journal of Pharmaceutical and Biomedical Analysis. 1998;8:871–875.
22. Murtaza G, Alikhan S, Shabbir A, Mahmood A, Farzana K, Malik NS, et al. Development of a uv-spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets. Scientific Research and Essays. 2011;6(2):417-421.
23. Purkar AJ, Balap AR, Jadhav SB, Chaudhari PD. Development and validation of uv spectrophotometric method for simultaneous determination of rosuvastatin calcium and aspirin in its pure and pharmaceutical dosage forms. International Journal of Pharmaceutical and Chemical Sciences. 2012;1(3):1008-1012.
24. Patta S, Afreen S, Tappa S, Nagarajan G, Gnanaprakash K. Simultaneous estimation of aspirin and omeprazole in bulk by uv-spectroscopy. Journal of Drug Delivery & Therapeutics. 2017; 7(3):87-91.
25. Ahmed M, Biswas MHU, Rahman MM, Sadik G. Development of a spectrophotometric method for the determination of aspirin in blood sample. The Sciences. 2001;1(2):61-62.
26. Dhudashia KR, Patel AV, Patel CN. Simultaneous uv spectrophotometric estimation of clotrimazole and beclomethasone dipropionate in their combined dosage forms by Q Ğ absorption ratio, dual wavelength and first order derivative method. Inventi Journal. 2012;(2):94–97.
27. Patel RD, Maheshwari DG. Dual wavelength spectrophotometric method for simultaneous estimation of torsemide and amiloride hydrochloride in their combined dosage form. Der Pharma Chemica. 2014;6(2):43-49.
28. Lotfy HM, Hegazy MA, Rezk MR, Omran YR. Comparative study of novel versus conventional two-wavelength spectrophotometric methods for analysis of spectrally overlapping binary mixture. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015;148: 328–337.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63616