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

Heba Zhdan¹ and Nazira Sarkis¹*

¹Department 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.

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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 λ₁ and λ₂ for determination. And the two wavelength 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 0.24 μg/mL and 0.82 μg/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 be7.13 μg/mL and 21.60μg/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;
1. INTRODUCTION

Rivaroxaban is 5-chloro-N-[(5S)-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).

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

![Fig. 1. The chemical structures of: A- rivaroxaban, B- aspirin](attachment:Chemical_Structures.png)
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**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Dual wavelength method</th>
<th>First derivative method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>RXN¹</td>
<td>ASP²</td>
</tr>
<tr>
<td>Wave lengths nm</td>
<td>∆A between 250, 286.44</td>
<td>∆A between 243.53, 259.2</td>
</tr>
<tr>
<td>Linearity-range(μg/ml)</td>
<td>2-12</td>
<td>40-240</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y=0.0552X-0.0001</td>
<td>Y=0.0022X+0.0452</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9996</td>
<td>0.9999</td>
</tr>
<tr>
<td>LOD μg/ml</td>
<td>0.24</td>
<td>7.03</td>
</tr>
<tr>
<td>LOQ μg/ml</td>
<td>0.82</td>
<td>22.01</td>
</tr>
<tr>
<td>*Accuracy (mean±RSD%)</td>
<td>100.39±0.28</td>
<td>99.72±0.37</td>
</tr>
</tbody>
</table>

¹ abbreviation of Rivaroxaban
² abbreviation of Aspirin

**Table 2. The accuracy data of the developed methods**

<table>
<thead>
<tr>
<th>Amt. of sample (μg/ml)</th>
<th>Amt. of drug added (μg/ml)</th>
<th>% Recovery (n=3) *</th>
<th>Dual wavelength</th>
<th>First derivative method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RXN⁷ ASP² RXN ASP</td>
<td></td>
<td>ASP RXN ASP</td>
<td>ASP RXN RXN</td>
</tr>
<tr>
<td>5</td>
<td>100 4 80</td>
<td>99.57 100.87 99.40 100.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100 5 100</td>
<td>99.75 99.73 100.13 100.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100 6 120</td>
<td>100.07 100.36 100.23 100.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>99.79±0.42 100.32±0.28 99.92±0.63 100.38±0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


