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Bioanalysis of the Rivaroxaban and Amlodipine in Dosage Forms and Spiked Plasma Using UPLC

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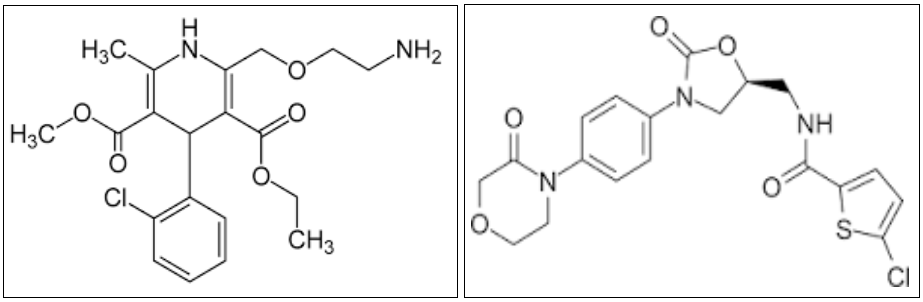
Abstract
The current research provides a bioanalytical technique for the analysis of definite co-administered drugs utilized for medication of nonvalvular atrial fibrillation. These studied drugs are rivaroxaban and amlodipine. The analysis was conducted by reversed-phase high-performance liquid chromatography utilizing Microsorb-MV 100-3 C18 column from Agilent Technologies (Netherlands; 100 mm 4.6 mm, 5 m) at room temperature with UV detection at 250 nm. Methanol, acetonitrile, & 10 mM sodium phosphate buffer (pH 3.6) made up the mobile phase, which had been pumped at a flow rate of 1.0 mL/min. Over 2 medicines' concentration range of 5–50 g/mL, the calibration curve had been linear. the proposed technique had been effectively used to analyze these medications in dose forms & in plasma samples that had been spiked.

Keywords: Rivaroxaban, Amlodipine, Dosage, UPLC

Introduction

Atrial fibrillation has been the most frequent cardiac arrhythmia. Nonvalvular atrial fibrillation may be a risk factor for stroke [1]. Aims of treatment incorporate prevention of rate & rhythm-related symptoms & the prevention of stroke & systemic emboli. Synergistic drugs can be used for antiarrhythmic or antihypertension therapy for keeping sinus rhythm &retain anticoagulation treatment to decrease the risk of stroke [2]. Combination therapy with an antihypertensive agent as amlodipine, and an Xa inhibitor as rivaroxaban improve anticoagulant activity in nonvalvular atrial fibrillation. Amlodipine has been 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5 pyridine dicarboxylic acid 3-ethyl 5-methyl ester (Figure1) [3]. It has been a synthetic dihydropyridine, approved by the FDA in 1987, It's a calcium channel blocker that has been generally used in the therapy of hypertension through the widening of blood vessels. Its salts, including besylate, mesylate, or maleate, can be present in formulations. White powdered amlodipine can be dissolved in organic solvents like ethanol, DMSO, & dimethyl formamide [4]. Studies for the detection of amlodipine in medications & biological fluids have been released, & one of the methods used was UV spectrophotometry [5], spectrofuorimetry [6], HPLC [7], UPLC [MS/MS [8], HPTLC [9]. Rivaroxaban has been known as 5-Chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4 morpholinyl)phenyl]-1, 3-oxzolidin-5-yl}methyl)-2-thiophene-carboxamide (Fig1). Rivaroxaban has been white to yellowish powder [10]. FDA approved it in 2011[4] Rivaroxaban has been an oral direct factor xanthine inhibitor. Factor Xa inhibition disrupts the blood coagulation cascade by preventing the generation of thrombin & growth of thrombi. In organic solvents (such as acetone, methanol, and polyethylene glycol), rivaroxaban has been very marginally soluble [11]. Studies for its determination had been described comprising UV spectrophotometry [12], spectrofuorimetry [13], HPLC [14], UPLC [15], LC-MS/MS [16], and HPTLC [17]. the goal of this research has been to develop a bioanalytical technique for the estimation of certain synergistic drugs used for the therapy of nonvalvular atrial fibrillation. studied drugs have been amlodipine & rivaroxaban. They are determined in dosage forms and spiked plasma.



Amlodipine Rivaroxaban

Fig 1: Structural formula of studied drugs

Instrument

With UV Agilent detector 1290DAD (Model: G4212A; Serial No. DEBAF04676, USA) set at 225nm & fluorescence Agilent detector 1260FLD (Model: G7121A; Serial No. DEAE300893, USA), separation had been carried out using Microsorb-MV100-3 C18 column (100mm × 4.6mm, 5µm; Agilent Technologies, Netherlands).

Materials & Reagents

Solvents used here had been of HPLC grade, while chemicals had been of analytical reagents grade. Kahira Pharmaceuticals & Chemical Industries (Cairo, Egypt) graciously provided the amlodipine. We gratefully received rivaroxaban from Medizen Pharmaceutical Industries in Alexandria, Egypt. Amlodipine® 10mg tablets and Andorivaban®2.5mg tablets were obtained from a local pharmacy. Acetonitrile and methanol had been bought from Sky Chem for lab supplies, in Alex, Egypt. Phosphate buffer had been arranged from sodium phosphate monobasic ortho anhydrous, disodium hydrogen phosphate & dist. water.

Column and Mobile Phase

Agilent Technologies (Netherlands) Microsorb-MV 100-3 C18 column (100mm × 4.6mm, 5µm) is employed. A combination of 50% methanol, 25% acetonitrile, and 25% phosphate buffer makes up the mobile phase. Orthophosphoric acid has been used to get pH to 3.6. After that, the mixture had been sonicated for thirty minutes. Afterward, a 0.45µm Millipore filter had been used to filter the translucent mobile phase that resulted. Mobile phase has just been made. To achieve a steady baseline, a mobile phase has been conducted for one hour before the column has been stabilized. The value that the column holds up to has been 1st deviation from the established baseline.

Standard Solutions

Amlodipine & rivaroxaban stock solutions had been made by dissolving five mg of medication in methanol & adding 50 mL of the same solvent to the volumetric flask. With the use of the mobile phase, these stock solutions had been further diluted to achieve a working concentration range of 5–50 µg/mL. When stored in the refrigerator, stock solutions remained stable for a full week. By elution of each medication separately, the stability of the stock solution has been examined. There are no signs that medications are breaking down.

Construction of Calibration Graph

By serially diluting stock solutions, working solutions with concentration ranges of five–fifty from 2 medicines have been created. below prior chromatographic conditions, aliquots of twenty µL had been injected (in duplicate) & eluted with the mobile phase. To determine the standard calibration curve, the drug's peak area vs concentration is plotted. Regression equation has been created then.

Analysis of Amlodipine & Rivaroxaban Tablets

Amlodipine ten mg & Xarelto ten mg pills had been weighed precisely, finely ground, & thoroughly combined. Amounts of powder aliquoted to equal ten mg of each of the 2 medicines had been placed into separate 100mL volumetric flasks. Eighty mL of methanol had been used to extract medicines, flasks had been then subjected to 20-minute sonication, filtered into100mL volumetric flask, & volume had been finished with methanol. To acquire working concentration ranges for each compound, aliquots of these solutions had been diluted with the mobile phase. 20 microliters had been injected (in duplicate), & mobile phase was used to elute them. Corresponding calibration curves or regression equations were used to define the necessary concentration.

Analysis of Amlodipine & Rivaroxaban in Spiked Plasma

Process of spiking plasma (blank matrix) with appropriate drug concentrations allowed for the construction of calibration curves for spiked plasma.

The sample plasma (blank matrix) was transferred in 1-milliliter aliquots.

into a series of centrifuge tubes that had been spiked with medications' operational concentrations (5, 10, & 50 µg/mL for amlodipine & rivaroxaban, respectively). Each tube received 5 milliliters of ethyl acetate before being centrifuged at 4000 rpm for twenty minutes. top organic layers had been removed, put in clean centrifuge tubes, & evaporated under nitrogen gas until dry. residues had been reconstituted in methanol, followed by completion in methanol, & then transferred to ten mL volumetric flasks. To get working concentrations, aliquots of these solutions had been diluted with the mobile phase. the mobile phase had been injected (in duplicate) into 20 microliters. related regression equations were used to determine the required concentration.

Results and Discussion

The high sensitivity of the technique makes it easy to define studied drugs in spiked plasma & for pharmacokinetic studies.

Drug peaks have been eluted at retention times of 3.2&6.5min for rivaroxaban &amlodipine respectively. peaks have been split (Figures 2, 3)and are well resolved with a resolution factor of 7.6.

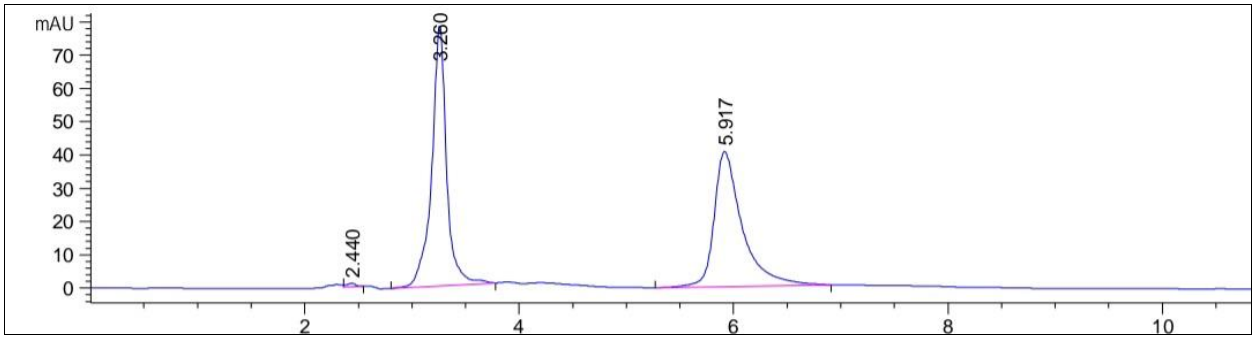


Fig 2: Chromatogram of studied drugs in pure form

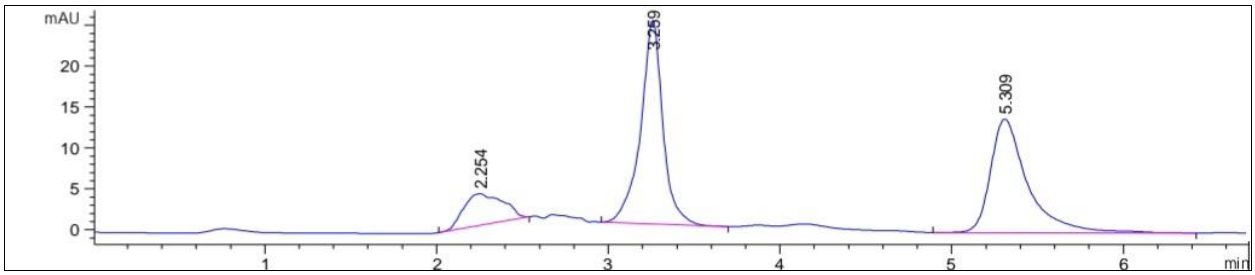


Fig 3: Chromatogram of studied drugs in spiked plasma

Ultraviolet Detection

The wavelength of 250 nm had been chosen because it offers good sensitivity for the identification of medications after multiple wavelengths were explored.

Optimization of Technique

It examined & optimized how experimental variables impact the chromatographic separation of medications. The sequential 1-by-1 method is used to optimize each experimental variable. These
variables like flow rate & mobile phase. To get the most theoretical plates & good resolution, variables have been optimized by adjusting each one individually whilst holding others constant. When it came to producing clear, symmetrical peaks, using phosphate buffer had been more effective than utilizing unbuffered samples. It investigated how pH affected the separation of analytes. Different pH levels (2, 3.6, 6 & 8) have been tested. It had been determined that pH 3.6 had been the most suitable option since it produced the best separation with the right amount of resolution and run time. Investigations into the relationship between flow rate & retention times revealed that a flow rate of one ml/min had been ideal for effective separation. Methanol, acetonitrile, and ten mM sodium phosphate buffer (pH 3.6) were used under ideal conditions, and the mobile phase had been pumped at a flow rate of 1.0 mL/min.

Analytical Validation

According to ICH rules, attributes of the analytical procedure's validation have been examined.

Linearity of Technique

The link between peak area & final drug concentrations resulted from refining circumstances, & it had been linear over ranges of 5 to 50 µg/ml. To determine linearity, 5 concentrations of each drug have been employed & analyzed twice. Linear regression of analysis of results gives the following equations:

Peak area ratio (amlodipine) = 61.63 C + 56.87, r^2=0.99
Peak area ratio (rivaroxaban) = 38.97 C + 26.12, r^2 =0.998

Where:

C = concentration (µg/mL),
r = correlation coefficient

There was a good relationship among peak area & concentration of each drug in the concentration range showed [18].

Accuracy

Over the defined range, the accuracy of the suggested procedure had been established. To evaluate assayed technique's dependability & repeatability. Measurement precision & accuracy are assessed by basic process. By using replicate analysis, the precision of the current technique had been investigated. 3 determinations had been examined for each concentration. 3 concentrations in the linearity range had been used to evaluate intra-day & inter-day precision on the same day & different days, respectively. Recovery% & RSD% computed values fell within an acceptable range (Table 1). suggested technique's great accuracy & precision were revealed by tiny, computed values of Recovery% & RSD% (Table 1), which also confirmed the technique's dependability.

Table 1: Validation parameters & outcomes achieved by RP-HPLC way recommended for the definition of amlodipine & rivaroxaban

Parameters	Amlodipine	Rivaroxaban
Measurement wavelength (nm)	250	
Linearity range (µg/ml)	5 – 50	
LOD	0.05	0.11
LOQ	0.15	0.32
Slope ± Sb	61.63 ± 23.99	38.97± 32.12
Intercept ± Sa	56.87 ± 0.94	26.12 ± 1.26
% RSD of Sb	1.51	1.499
Correlation coefficient	0.99	0.998

Repeatability

Nine results covering the process's stated range (3 concentrations/3 replicates each) were used to evaluate repeatability (intraday precision). Calculated as percentage relative standard deviation. Table 2 provided a summary of the findings. The low percentage of RSD in outcomes indicates good precision.

Table 2: Intra-day & inter-day precision & accuracy to define studied drugs using the proposed technique

Rivaroxaban (µg/mL) Intraday precision & Accuracy		Mean percent Recovery	RSD
Added conc	Found conc		
5	5.1	102	0.26
20	20.03	100.3	0.12
50	49.93	99.5	0.42
Intraday precision & accuracy			
Added conc	Found conc		
5	5.07	101.3	0.32
20	20.1	100.1	0.26
50	50.17	100.8	1.02

⇩

Amlodipine (µg/mL) Intraday precision & Accuracy		Mean percent Recovery	RSD
Added conc	Found conc		
5	4.9	99.3	0. 31
20	20.06	100.6	0.49
50	50.1	100.5	0.98
Intraday precision & accuracy			
Added conc	Found conc		
5	5.06	100.2	0.15
20	19.93	99.3	0.17
50	50.03	100.15	0.59

Specificity

Throughout the technique's validation, a specificity study had been made. A mixture of solutions including active dosage of investigated medicines is administered. For rivaroxaban & amlodipine, drug peaks had been eluted at retention periods of 3.2 and 6.5 min, respectively.

Limits of Detection & Quantification

Based on the calibration curve, limits of detection & quantification had been established. LOD & LOQ had been determined using formulas LOD = 3.3Sa/b & LOQ = 10Sa/b, where Sa represents the regression line's standard deviation & b represents the calibration curve's slope. For amlodipine & rivaroxaban, LODs are 0.05 & 0.11, respectively. Amlodipine & rivaroxaban had LOQs of 0.15 & 0.32, respectively.

Robustness

The robustness of the technique had been studied according to ICH guidelines. The technique had been done below small differences like pH of the mobile phase (3.6±0.2), mobile phase composition (methanol50**%**: acetonitrile25**%**: sod phosphate buffer 25**%** v/v/v± 3ML of all solvent components), and flowrate(0.8–1.2 mL). Small %RSD < 2 values were obtained [19].

System Suitability

To make sure the entire testing system is appropriate for the planned application, tests had been done. Several theoretical plates, resolution, & capacity factors were all tested for system compatibility. The outcomes have been shown in Table (3).

Table 3: System suitability tests of HPLC technique offered for determination of amlodipine & rivaroxaban

PH	Number of theoretical plates		Capacity factor K`		Resolution factor Rs
	Amlodipine	Rivaroxaban	Amlodipine	Rivaroxaban	
3	1130	835	0.48	1.98	7.62
3.6	1241	879	0.48	1.98	7.6
4	1115	840	0.5	2	7.6
5	1109	832	0.52	2.09	7.6
6	1100	828	0.53	2.2	7.02
7	1097	817	0.53	2.4	7.02

Methanol conc	Number of theoretical plates		Capacity factor K`		Resolution factor Rs
	Amlodipine	rivaroxaban	Amlodipine	rivaroxaban	
10 %	1000	708	1.1	2.5	7.75
25 %	1100	760	1.07	2.35	7.69
30 %	1162	802	1.02	2.2	7.66
50 %	1241	874	0.48	1.98	7.6
60 %	1150	811	0.42	1.88	7.3

Acetonitrile conc	Number of theoretical plates		Capacity factor K`		Resolution factor Rs
	Amlodipine	rivaroxaban	Amlodipine	rivaroxaban	
10 %	1010	701	0.53	2.1	7.36
25 %	1241	874	0.48	1.98	7.6
30 %	1141	786	0.41	1.95	7.69
50 %	1015	772	0.38	1.93	7.53
60 %	987	733	0.37	1.9	7.5

Buffer conc	Number of theoretical plates		Capacity factor K`		Resolution factor Rs
	Amlodipine	rivaroxaban	Amlodipine	rivaroxaban	
10 %	1100	840	0.49	2	7.82
25 %	1241	874	0.48	1.98	7.6

30 %	1045	861	0.47	1.96	7.58
50 %	1002	840	0.42	1.8	7.55
60 %	980	821	0.38	1.78	7.48

Stability

By reassessing sample solutions, it had been confirmed that they remained stable at room temperature (twenty-five° C) for twenty-four hours following preparation. There had been no sign that medications had broken down in any way.

Applications

Application to Dosage Forms

The suggested technique worked well for estimating the dosage of investigated medications in tablet form. outcomes in Table 5 matched those obtained using standard USP techniques quite well (USP, 2007). Comparing proposed techniques to approved techniques is sensitive.

Application to Spiked Biological Fluids

With the high rate of recovery, the bioanalytical technique's high sensitivity allows for the identification of pharmaceuticals in biological fluids (Table 4, Figure 4 depict chromatograms of spiked plasma samples comprising 2 medications analyzed under ideal conditions).

Table 4: Application of the suggested technique to define studied drugs in spiked plasma

Amlodipine Conc. added % Found (µg/mL)		**Rivaroxaban** Conc. added % Found (µg/mL)	
100.6	5	99.3	5
99.6	20	100.6	20
99.5	50	101.3	50
99.9±0.63	mean ± SD	100.4±0.94	mean ± SD

Conclusion

Co-administered medications amlodipine & rivaroxaban, which have been used to treat nonvalvular atrial fibrillation, have been simultaneously analyzed by a suggested bioanalytical approach. The current inquiry is trustworthy to monitor the concentration of studied medications in studied case plasma due to the great sensitivity of the procedure. It may also be prolonged for pharmacokinetic investigations.

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