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In vitro Drug-Drug Interaction Studies of Apixaban with Atorvastatin by HPTLC Method

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Abstract

Poly-pharmacy of cardiovascular drugs is a common cause of a few unexpected drug-drug interactions (DDI) which may lead to the occurrence of the adverse effects of the drugs co-administered. The High Performance Thin Layer Chromatography method was adopted for the analysis of apixaban and atorvastatin. The method validation for the individual drugs was carried out according to the ICH guidelines. The validated method was applied for the *in vitro* drug-drug interaction study is at the biological pH of stomach at 4.0, of blood at 7.4 and intestinal condition at 9.0. The aim of the study is to evaluate the effect of atorvastatin under the simulated conditions of the human body. The *in vitro* drug-drug interaction studies suggest that the drug apixaban does not have any profound change at pH 4 and 9 when present with atorvastatin. When apixaban and atorvastatin are present at a pH 7.4 the significant variation in the detector response is indicative of the decrease in the level of apixaban.

Keywords: Drug interaction; Apixaban; Atorvastatin; HPTLC method.

Introduction

Apixaban, 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide [1], is indicated to reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. Its molecular formula is $C_{25}H_{25}N_5O_4$, which corresponds to a molecular weight of 459.5.

Apixaban appears as a white-to-pale yellow, non-hygroscopic crystalline powder, with an aqueous solubility of 0.028 mg/mL at 24°C. The reported methods of estimation of apixaban by spectroscopic [2,3] and liquid chromatographic methods [4,5] were found in the literatures. The other methods reported were surveyed for the selection of research work [6-8]. Till date there are no *in vitro* interaction studies reported for apixaban by HTPLC. Further, apixaban is not official in any pharmacopeia.

Atorvastatin calcium is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A d(HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis.

Atorvastatin calcium is [R-(R*,R*)]-2-(4-fluorophenyl)-ß, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. The empirical formula of atorvastatin calcium is $(C_{33}H_{34}\ FN_2O_5)2Ca{\bullet}3H_2O$ and its molecular weight is 1209.42.Atorvastatin calcium is a white to off-white crystalline powder that is insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol [9,10].

The objective of the present work is to develop a method and validate as per the ICH parameters [11]. The developed method is applied for its determination as single drug for apixaban and atorvastatin and in combination. The *in vitro* drug drug interaction studies between apixaban and atorvastatin indicates the potential interaction between the drugs at pH 7.4.

Materials and Methods

The organic solvents like methanol, toluene, divchloromethane and tetrahydrofuran was of AR grade used for solution preparation and mobile phase system were procured from SD Fine Chemicals Ltd, Mumbai. The Active Pharmaceutical Ingredient Apixaban was procured from Sigma Aldrich, Germany. The tablet formulations of Apixaban and atorvastatin was procured from the local pharmacy in Coimbatore. The drug atorvastatin selected for interaction was procured from Yarrow Chem Products, Mumbai.

The HPTLC method was developed using precoated silica gel plate GF 254, Camag UV chamber for spot identification, Camag linomat 5 applicator for sample application, 20 x 20 cm and 10 x 10 cm chamber for plate development and Camag TLC Scanner for spot detection with WinCats Software. The Digital pH meter MK VI was used for the pH measurement of the buffer and drug solutions.

Chromatographic conditions

The solvent system consisting of Toluene: Dichloromethane: Tetrahydrofuran in the ratio of 2:2:6(v/v/v) with two drops of glacial acetic acid was found to separate the peaks of apixaban and atorvastatin. The wavelength selected for the study was 279 nm with chamber saturation time of 20 minutes.

Stock solution of apixaban and atorvastatin in the concentration of 1000 mcg/ml was prepared with methanol. The working standard was prepared in the concentration of 100 mcg/ml with the same.

Validation of HPTLC method for Apixaban and atorvastatin

Linearity and range: The method shows a linear correlation for apixaban between 200-700 ng/spot (Figure 1) and 400-1400 ng/spot for atorvastatin (Figure 2). The peak area was subjected to regression analysis by the least square method. The results are given in Table 1. The correlation coefficient was more than 0.99 for both the drugs.

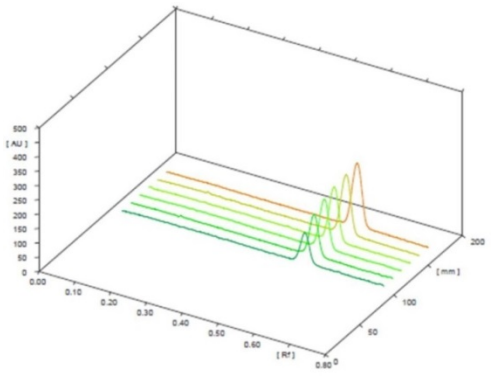

Figure 1: 3D Densitogram of apixaban.

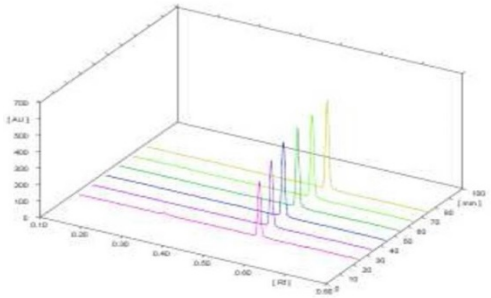

Figure 2: 3D Densitogram of atorvastatin.

Apixaban			Atorvastatin		
Conc ng/ spot	Rf value	Peak Area	Conc ng/ spot	Rf value	Peak Area
200	0.54	2574	400	0.68	3324
300	0.54	3294	600	0.69	4489
400	0.54	4014	800	0.69	5989
500	0.55	4567	1000	0.69	7262
600	0.55	5138	1200	0.7	7871
700	0.56	5587	1400	0.7	8850

Table 1: Linearity data.

Intraday and interday precision: The repeatability of the measurements were studied on the same day. The interday precision was carried on three consecutive days. The standard deviation and % relative standard deviation was calculated. The % RSD was found to be below 1 indicating the method precise.

Accuracy: The recovery of the standard drug was evaluated at two different levels of 50% and 100% for the tablet formulation for both the drugs. The % recovery of the standard drug from the solution was calculated and found to be 93.80 and 97.40% for the selected brand xarelto 2.5 mg tablet. For the Atorva 5 mg tablet the recovery was 101.0% and 99.8% for the two levels carried out.

Robustness and ruggedness: The experimental parameters varied intentionally were the chamber saturation time by ± 2 minutes and mobile phase composition by ± 0.1 ml. The peak area recorded were found to show no notable variation from the fixed parameters.

Stability of the plate: The plate was scanned at every two hours interval. The peak area recorded was found to be same till 10 hours after the values varied. The results are mean of six determinations.

LOD and LOQ: The limit of detection found by the detector was found to be 50 ng/spot for apixaban and 100 ng/spot for atorvastatin. The limit of Quantitation was found to be 100 ng/spot and 200 ng/spot for apixaban and atorvastatin respectively.

Analysis of formulations: The Xarelto tablets 2.5 mg and the atorva 5 mg tablets were analysed for the percentage purity using the fixed chromatographic conditions. The amount present in the tablet was found to be 103 % for apixaban and 101.2 % for atorvastatin. The statistical estimation of six determinations were less than 1 for the % RSD value for both drugs.

In vitro drug-drug interaction studies

Preparation of buffer solutions: The pH 4.0 buffer was prepared in distilled water by weighing 3.725 g of potassium chloride in 1000 ml water. The pH was adjusted upto 4.0 using 0.1 M HCl. The buffer pH 7.4 was prepared by weighing accurately 0.6 g of potassium dihydrogen ortho-phosphate, 6.4 g of disodium hydrogen orthophosphate and 5.85 g of sodium chloride in 1000 ml of HPLC grade water. The pH 7.4 was adjusted using 0.1 M HCl. The pH 9.0 buffer was prepared by weighing 4.98 g of ammonium chloride and dissolved in water after which the volume was made up to 1000 ml with the same. The pH was adjusted with ammonia solution up to 9.0.

Preparation of solution: A 500+1000 mcg/ml of mixture stock solution for apixaban and atorvastatin was prepared in methanol. The mixture of apixaban and atorvastatin working solution was prepared by diluting to a concentration of 50+100 mcg/ml with buffer solution. The volume spotted for the analysis was 10 µl of the mixture solution.

Method adopted for *in vitro* drug-drug interaction studies: The mixture of drug apixaban and atorvastatin in the buffer solution was kept on a thermostat at 37°C. The isolation and detection of apixaban and atorvastatin at the selective Rf value for the peak area and change in the response were tabulated. The sampling was at an interval of one hour till 5 hours for the three buffer pH. The blank buffer pH 4.0, 7.4 and 9.0 were spotted to identify any interference with the drug peaks. The spots of each mixture were studied for the peak area, Rf value and the changes at regular intervals (Figures 3-5). The Table 2 illustrates the change in detector response from zero hours to five hours.

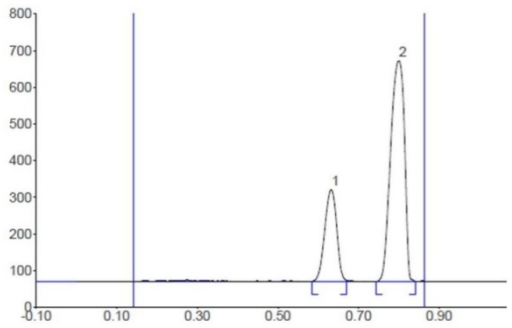

Figure 3: HPTLC chromatograms of apixaban+atorvastatin mixture at 5 hours at pH 4.0.

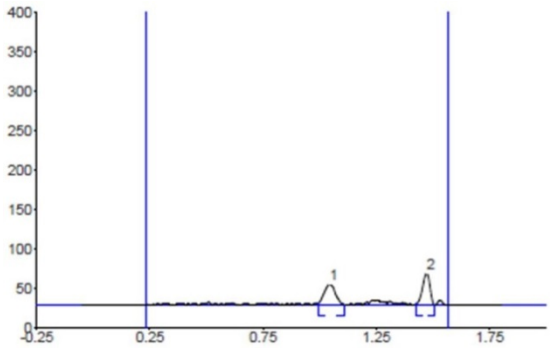

Figure 4: HPTLC chromatograms of apixaban+atorvastatin mixture at 5 hours at pH 7.4.

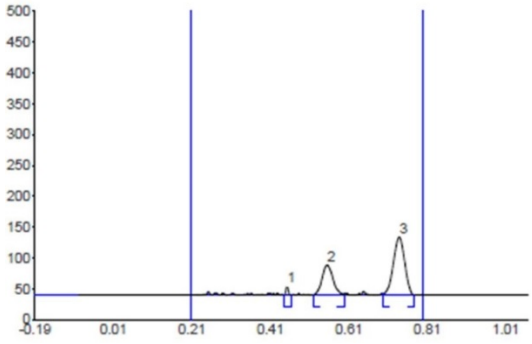

Figure 5: HPTLC chromatograms of apixaban+atorvastatin mixture at 5 hours at pH 9.0.

pH	Time Hr	Mixture		
		Rf value	Peak area	% Changes in peak area
4		0.58	6391	-
	0	0.78	17718	-
	5	0.63	6727	5.27
		0.8	18592	4.92
7.4	0	0.96	1080	-
		1.4	893	-
	5	1.04	602	44.25
		1.48	600	32.81
9	0	0.54	1141	-
		0.73	1543	-
	5	0.57	1110	2.82
		0.76	2041	32.27

Table 2: *In vitro* drug-drug interaction studies of apixaban with atorvastatin.

Results and Discussion

The HPTLC method was developed using different organic solvents based on the polarity and solubility of the drugs. The proportional change in the ratio of Tetrahydrofuran had a significant effect in the Rf value of the spots. So the solvent system made up of Toluene: Dichloromethane: Tetrahydrofuran in the ratio of 2:2:6 has been selected. The solvent composition carried the drug apixaban and atorvastatin to the same distance on the plate. So to have an acceptable separation between the two drugs selected two drops of glacial acetic acid was added which resulted in good resolution of both the drugs. The experimental criterion such as the distance travelled by the solvent front, chamber saturation time and the peak characteristics including the peak symmetry, area and Rf value were fixed after numerous trials.

The method developed was validated for the linearity, range, accuracy, interday precision, intraday precision, ruggedness, robustness, limit of detection and limit of quantification [11].

The *in vitro* drug-drug interactions carried out highlights the eventual changes when both are present together in various biological fluids at the selected pH. The conditions of study like the time of interaction was selected as the time to reach the peak plasma concentration is four hours for apixaban [1]. The Rf value of the drug apixaban in solution of the three pH 4.0, 7.4 and 9.0 were run separately and the results were interpretated. At pH 4.0 and 9.0 the Rf value was found to be at .0.58 and 0.54 at zero hours and the changes in the responses were less than 10%. At the pH 7.4 the peak area changes were 44% for apixaban and 33% for atorvastatin. The peak area at zero hours was compared with the peak areas at the particular time of analysis. The considerable changes in the peak area were calculated and tabulated in Table 2.

Both the selected drugs are marketed in the potent dose of less than and equal to 10 mg. The drug apixaban belongs to the class of anticoagulants and atorvastatin is a lipid lowering agent. Co-administration of both the class of drugs is a common regime. The research work suggests no interactions between apixaban and atorvastatin at pH 4 and 9 whereas the responses at a pH 7.4 indicate decrease in the drug concentration of apixaban.

Conclusion

The method developed and validated can be applied for the routine analysis of Apixaban and atorvastatin. The interaction method is an insight into the safe administration of apixaban and atorvastatin.

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