New Validated Method for the Estimation of Pioglitazone and Rosiglitazone Using RP-HPLC

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Authors' contributions

This work was carried out in collaboration between both authors. Author KR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SR managed the analyses of the study, managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

New, simple and economical high pressure liquid chromatography method has been developed for the simultaneous quantification of Pioglitazone and Rosiglitazone.By using Waters HPLC e-2695 quaternary pump with a PDA detector of 2998 instrument the chromatographic separation of Pioglitazone and Rosiglitazone was achieved on the column of Inertsil ODS (150x4.6mm, 3.5 µ) using an isocratic elution with a buffer containing 0.1percentformic acid and acetonitrile at a rate of 30:70 as a mobile phase with a flow rate of 1 ml/min at ambient temperature. A detector wavelength of 261 nm utilizing the PDA detector were given in the instrumental settings. The linearity was studied between the concentration range of 3-45 µg/ml of Pioglitazone and 1-15 µg/ml of Rosiglitazone were injected.The plotted calibration curves were linear with a regression coefficient of $R^2 > 0.999$, indicates that the linearity was with in the limit. As a part of method validation the parameters like specificity, linearity, accuracy, ruggedness, robustness were determined and the results were found to be within the allowable limit. The method developed was found to be applicable to routine analysis and to be used for the measurement of both active pharmaceutical ingredients (i.e, Pioglitazone and Rosiglitazone).Validation of the proposed method was carried out according to an International Conference on Harmonization (ICH) guidelines.

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Since, there is no HPLC method reported in the literature for the estimation of Pioglitazone and Rosiglitazone, there is a need to develop quantitative methods under different conditions to achieve improvement in specificity, selecivity etc.

1. INTRODUCTION

Pioglitazone, marketed with a trade name Actos, among others, is an anti-diabetic drug used to treat type 2 diabetes [1, 2]. It can be utilized with metformin, sulfonylurea[3] or insulin [4]. Use of exercise and diet is advised. It is not recommended for type 1 diabetes[5]. It's taken by the mouth. Usual side reactions include headaches, muscle pain[6], inflammation[7] of the throat, and swelling. Severe side reactions can include cancer of the bladder [8,9] hypoglycaemia[10], heart failure [11] and osteoporosis [12]. Usage not recommended during pregnancy or breast-feeding. It is in the class of thiazolidinedione (TZD)[13] and functions by bettering the sensitivity of tissues to insulin.

Rosiglitazone (trade name Avandia) is an anti-diabetic drug in the thiazolidinedione class[14]. It acts as an insulin sensitizer, binding to PPAR in fat cells and producing the cells more sensitive to insulin. It is sold by the pharmaceutical firm GlaxoSmithKline (GSK) as a stand-alone drug or for use in conjunction with metformin or glimepiride. However, following a meta-analysis in 2007, which related drug utilized to rised the risk of heart attack [15,16].

Pioglitazone and rosiglitazone undergo extensive phase I metabolism. Although pioglitazone and rosiglitazone have typically given negative results when assessed in standard batteries of genotoxicity assays, exceptions have been noted. Certain pioglitazone metabolites and rosiglitazone have given positive results in assays in the mouse lymphoma cells; pioglitazone increased the levels of chromosomal aberration, sister chromatid exchange, and 8-oxodeoxyguanosine in human peripheral blood lymphocytes; and both pioglitazone and rosiglitazone gave positive results in comet assays in liver cells and peripheral blood lymphocytes from rats.

There are some HPLC methods [17-24] reported in the literature, but these methods are developed only for individual analysis of Pioglitazone and Rosiglitazone in bulk and formulation studies. The developed HPLC method was utilized for the estimation of the combined drugs by *in vitro* method. Fig. 1 shows the chemical structures of Pioglitazone and Rosiglitazone.

2. MATERIALS AND METHODS

2.1 Chemicals

Acetonitrile, HPLC-grade formic acid, water were purchased from Merck India Ltd, Mumbai, India. APIs of Pioglitazone and Rosiglitazone standards were procured from Glenmark,Mumbai.

2.2 The Instrumentation

Waters alliance liquid chromatography (model e-2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study.

2.2.1 Preparation of buffer

1 ml of formic acid is dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

2.2.2 Chromatographic conditions

The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% formic acid and Inertsil ODS column (150x4.6 mm, 3.5 µ) column with a flow rate of 1 ml /min.
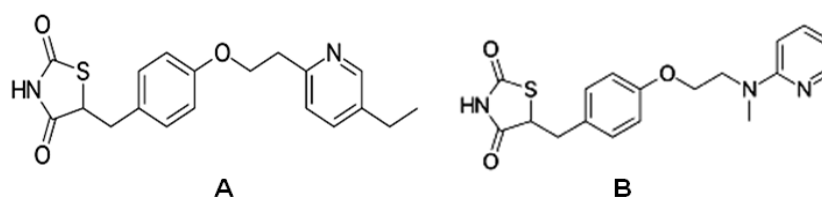

Fig. 1. Chemical structure of (a) pioglitazone (b) rosiglitazone

2.2.3 Diluent

Water and Acetonitrile in the ratio (50:50) is used as diluent.

2.2.4 Preparation of the standard stock solution

For standard stock solution preparation, add 70ml of diluents to 30mg of Pioglitazone and 10 mg of Rosiglitazone taken in a 100 ml volumetric flask and sonicate for 10 minutes to fully dissolve the contents and then make up to the mark with diluent.

2.3 Preparation of Standard Solution

5 ml of solution is drawn from the above normal stock solution into a 50ml volumetric flask and diluted up to the level.

3. RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide a good performance the chromatographic conditions were optimized.

3.1 Method Optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally 0.1% formic acid buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as C_8, C_{18} phenyl and amino,inertsil ODS columns were tested. From these trials the peak shapes were relatively good with a Inertsil ODS column of 150 x 4.6mm, 3.5 µ with a PDA detector. The mobile phase flow rate has been done at 261nm in order to obtain enough sensitivity. By using above conditions we get retention times of Pioglitazone and Rosiglitazone were about 2.770 and 5.118 min with a tailing factor of 1.02& 1.05. The number of theoretical plates for Pioglitazone and Rosiglitazone were 4132,7065 which indicate the column's successful output the % RSD for six replicate injections was around 0.18% and 0.26%, the proposed approach suggests that it is extremely

precise. According to ICH guidelines, the established method was validated.

3.2 Method Validation

The optimized RP-HPLC validated method according to ICH guidelines in terms of system suitability, linearity, accuracy, precision and robustness.

3.3 System Suitability

Device suitability was performed by injecting standard solution containing 30 µg/ml of Pioglitazone and 10 µg/ml of Rosiglitazone in six replicates. The results show that the machine fitness parameter is within the limit provided by ICH. The results were shown below. Table 1 gives the results of system suitability and see the Fig. 2 for standard chromatogram.

3.4 Specificity

There was no interference from blank at the retention time of Pioglitazone and Rosiglitazone. This proves the technique is specific. See the Fig. 3 for blank chromatogram.

3.5 Linearity

Linearity was calculated by plotting a calibration curve of the peak area against its respective concentration, linearity was determined. From this calibration curve, it was noticed that the curve was linear between the range of 3-45µg/ml of Pioglitazone and 1-15µg/ml of Rosiglitazone. The regression equations for calibration curve was Y=117695.97x+ 14774.06 (R^2=0.99976) for Pioglitazone and Y= 142819.95x+41820.07 (R^2=0.99922) for Rosiglitazone respectively. Linearity results were given in Table 2 and the calibration plots were in Fig. 4.

3.6 Accuracy

The accuracy of the system was achieved by measuring the recovery experiments at three stages (50 percent, 100 percent and 150 percent). APIs with concentrations of 15, 30 and 45µg/ml of Pioglitazone and 5, 10 and 15µg/ml of Rosiglitazone were prepared. For each spike stage, the test solution was injected three times and the test was performed according to the test

process. The recovery results were similar to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and relative standard deviations were determined. Recovery values shown within the desired range were correct. The results are summarized below. Accuracy findings have been shown in Table 3.

Table 1. Results of system suıtabılıty

System suitability parameter	Acceptance criteria	Drug name	
		Pioglitazone	**Rosiglitazone**
USP Plate count	NLT 2000	4132	7065
USP Tailing	NMT 2.0	1.02	1.05
USP Resolution	NLT 2.0	-	11.23
% RSD	NMT 2.0	0.49	0.92
Retention Time	NLT 2.0	2.770	5.118

Table 2. Lınearıty results

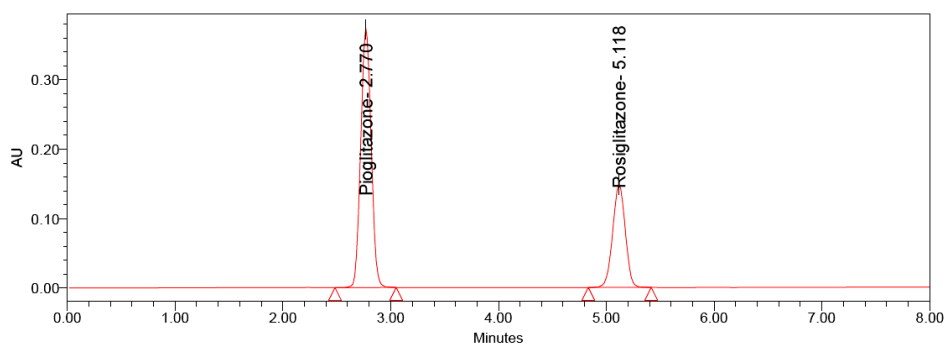| S. No.	Pioglitazone		Rosiglitazone	
	Conc. (µg/ml)	**Area**	**Conc. (µg/ml)**	**Area**
1	3.00	356890	1.00	177169
2	7.50	921356	2.50	418184
3	15.00	1805500	5.00	788226
4	22.50	2611265	7.50	1133741
5	30.00	3616456	10.00	1455465
6	37.50	4371404	12.50	1860828
7	45.00	5325525	15.00	2141815
Slope	117695.97		142819.95	
Intercept	14774.06		41820.07	
CC	0.99976		0.99922	

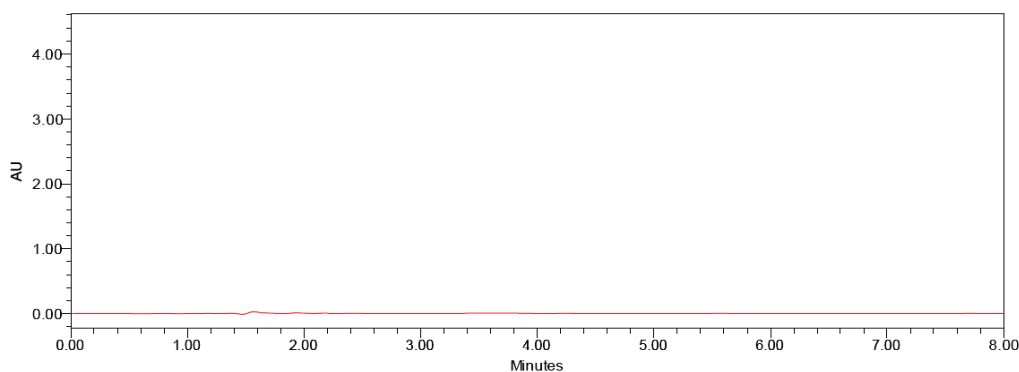**Fıg. 2. Chromatogram of standard**
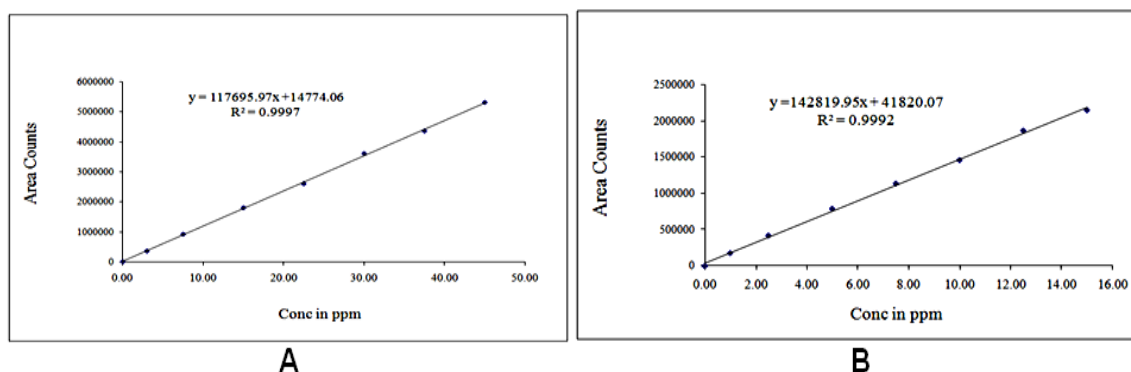

Fıg. 3. Chromatogram of blank

Fıg. 4. Calıbratıon plots of (a) pıoglıtazone (b) rosıglıtazone

Table 3. Results of accuracy

S. No.	% Level	Pioglitazone % Recovery	Rosiglitazone % Recovery
1	50	99.63	99.56
2	100	99.78	99.23
3	150	98.69	100.45

3.7 Precision

The precision of the analytical technique is the degree of proximity of the sequence of measurements obtained from multiple homogeneous mixture samplings. The accuracy of the process of the drugs were calculated by injection of six individual determinations of Pioglitazone (30 µg/ml) and Rosiglitazone (10µg/ml). Method precision results were shown in Table 4 and method precision chromatogram was shown in Fig. 5.

Table 4. Results of ıntraday precısıon

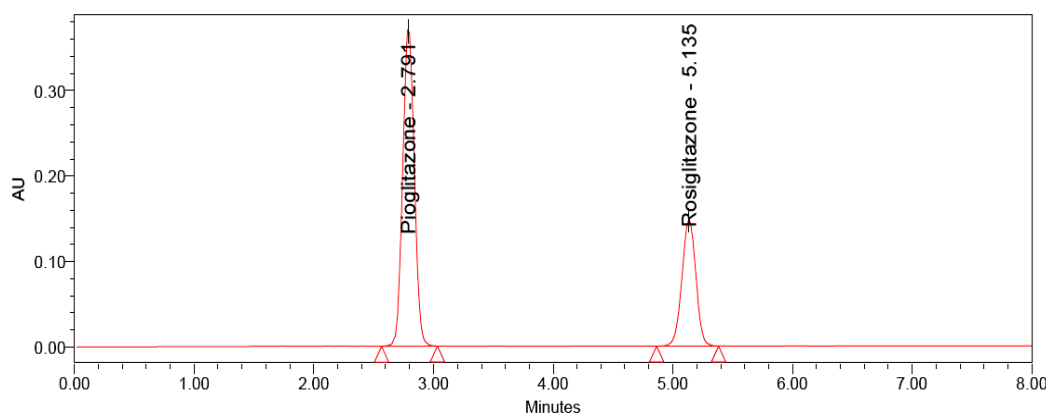| S. No.	Pioglitazone			Rosiglitazone		
	Conc. (µg/ml)	Area	% Assay	Conc. (µg/ml)	Area	% Assay
1	30	3652487	99.65	10	1436527	100.42
2		3625148	99.68		1442359	100.26
3		3695284	99.37		1425863	99.93
4		3687452	100.04		1476324	100.18
5		3602541	99.98		1452638	99.48
6		3636326	99.76		1475842	99.87
% RSD	0.99			1.44		

Fıg. 5. Chromatogram of method precısıon

3.8 Intermediate Precision

Six replicates of the standard solution were analyzed bydifferent researchers and different tools were checked on separate days. The peak regions used to assess the average percent of RSD values have been determined. The findings are shown in the Table 5.

3.9 LOD and LOQ

LOD and LOQ were determined separately using the calibration curve technique. The LOD and LOQ of the compound were measured using the developed RP-HPLC method by injecting lower and lower concentrations of the standard solution. The LOD and LOQ concentrations and their s/n values of Pioglitazone and Rosiglitazone were represented in the following table 6 and the LOD, LOQ chromatograms were shown in Fig. 6.

3.10 Robustness

The conditions of the experiment was designed to measure the robustness of the intentionally changed conditions such as flow rate, organic percentage in mobile phase. Results of robustness were represented in Table 7.

3.10.1 Degradation studies

Pioglitazone and Rosiglitazone standard was subjected to various conditions of forced degradation in order to induce partial degradation of the compound. Forced degradation experiments have been performed to establish that the process is acceptable for degradation materials. In addition the studies include information on the condition under which the drug is unstable, such that the steps are also taken during formulation to prevent possible instabilities. Degradation results were given in Table 8.

3.10.2 Acid degradation

1 ml of standard stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 1N HCl and left it for 15 min. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark.

3.10.3 Alkali degradation

1 ml ofstandard stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 1N NaOH and left it for 15 min. After 15 min add 1 ml of 1N HCl and make up to the mark.

3.10.4 Peroxide degradation

1 ml of standard stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 30% hydrogen peroxide solution and make upto the mark with diluents.

3.10.5 Reduction degradation

1 ml of standard stock solution was moved to a volumetric flask of 10 ml and add 1 ml of 30% sodium bi sulphate solution and make upto the mark with diluents.

3.10.6 Thermal degradation

The standard solution was set in an oven at 105°C for 6 hrs. The resultant solution was injected into HPLC system.

Table 5. Inter-day precısıon results

S. No.	Pioglitazone			Rosiglitazone		
	Conc.(µg/ml)	Area	% Assay	Conc. (µg/ml)	Area	% Assay
1	30	3652415	100.02	10	1475821	99.68
2		3626352	100.14		1462345	99.23
3		3642157	100.23		1478549	100.14
4		3639568	99.78		1478562	100.42
5		3675423	100.43		1436521	99.15
6		3621232	100.05		1432658	99.36
%CV	0.54			1.45		

Table 6. Lod and loq results

Pioglitazone				Rosiglitazone			
LOD		LOQ		LOD		LOQ	
Conc. (µg/ml)	s/n	Conc. (µg/ml)	s/n	Conc. (µg/ml)	s/n	Conc. (µg/ml)	s/n
0.038	6	0.124	26	0.013	3	0.041	24
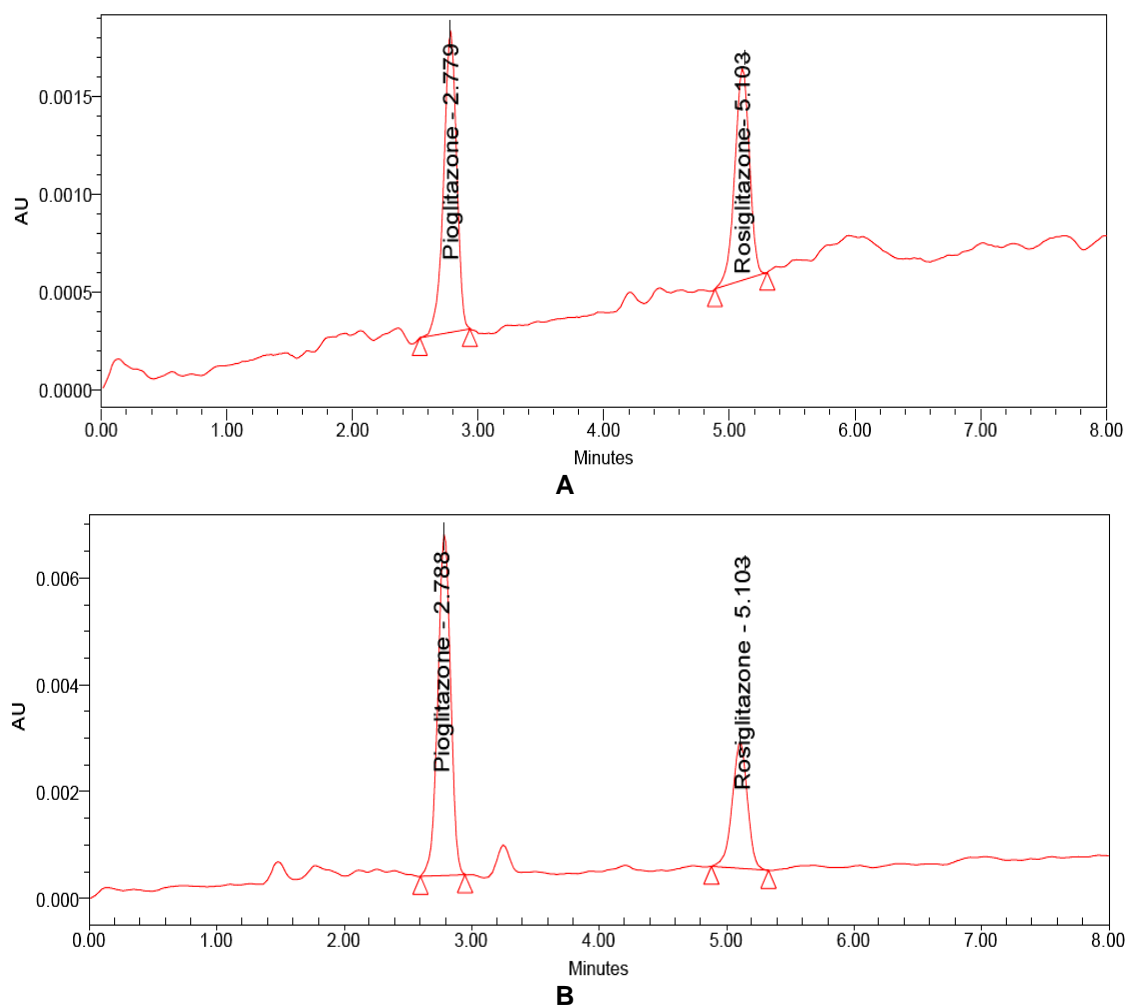
Fıg. 6. Chromatogram of (a) lod (b) loq

Table 7. Robustness results

Parameter name	% RSD	
	Pioglitazone	**Rosiglitazone**
Flow rate (0.8 ml/min)	0.37	1.01
Flow rate (1.2 ml/min)	1.24	0.87
Org Plus (66:34)	1.38	0.93
Org Minus (54:46)	0.59	0.74

Table 8. Forced degradatıon results

Degradation condition	Pioglitazone				Rosiglitazone			
	RT	Area	% Assay	% deg	RT	Area	% Assay	% deg
Control deg	2.787	3622125	99.9	0.1	5.111	1425178	99.9	0.1
Acid deg	2.791	3082478	85.1	14.9	5.135	1212437	85.1	14.9
Alkali deg	2.786	3071732	84.8	15.2	5.139	1220878	85.7	14.3
Peroxide deg	2.796	3127824	86.4	13.6	5.132	1240941	87.1	12.9
Reduction deg	2.793	3167851	87.5	12.5	5.128	1264479	88.8	11.2
Thermal deg	2.791	3208712	88.6	11.4	5.124	1280462	89.9	10.1
Hydrolysis deg	2.789	3220793	89	11	5.141	1256987	88.2	11.8

3.10.7 Hydrolysis degradation

1 ml of standard stock solution was moved to a volumetric flask of 10 ml and add 1 ml of HPLC water and make up to the mark with diluents.

4. CONCLUSION

This method described the quantification of Pioglitazone and Rosiglitazone as per ICH guidelines. The evolved technique was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of drug preparation and reproducibility data are satisfactory. The evolved chromatographic method can be effectively applied for regular investigation of the drugs (Pioglitazone and Rosiglitazone) in combined dosage form.

DISCLAIMER

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

CONSENT

This manuscript not published at any other journals.

ETHICAL APPROVAL

We are not performing any clinical trials in this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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