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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF EDOXABAN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

***S DIVYA TEJA. BANDA[1], Dr. MOHD JAVED NAIM [2]**

[1]Research Scholar, Bhagwant University, Ajmer, Rajasthan, INDIA-305004
[2]Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan, INDIA-305004.
*Corresponding Author

Abstract

RP-HPLC method for estimation of Edoxaban in bulk and pharmaceutical dosage forms was obtained with Hypersil ODS C18 (100mm×4.6 mm, 5µm particle size); Shimadzu LC-20AT Prominence HPLC system, equipped with SPD 20A detector and mobile phase contained a mixture of Potassium di-hydrogen phosphate (p^H adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v) was delivered at a flow rate of 1 mL/min. Quantitation was attained at 230 nm depends on peak area. The retention time of Edoxaban was 3.677 min. Linearity was established for Edoxaban in the range of 10.5-18 µg/mL with correlation coefficients (r=0.999) and the percentage recoveries were between 99.74 %-100.70 % for Edoxaban respectively. The RSD % values of accuracy for Edoxaban were found to be < 2 %, which indicate accuracy of the proposed method. The RSD % values of precision were found to be 0.121% for Edoxaban respectively and for ruggedness were found to be 0.43% and 0.94 % for Edoxaban respectively, reveal that the proposed method is precise. LOD values were found to be 0.03 µg/mL for Edoxaban and LOQ values were found to be 0.09 µg/mL for Edoxaban. The RSD % values of robustness studies were found to be < 2 %, which indicate robustness of the proposed method. These reports show that the proposed method was accurate and precise for determination of Edoxaban in bulk and pharmaceutical combined dosage forms. The developed method is simple, precise, and accurate. Hence, the RP-HPLC method can be applicable for the routine analysis of Edoxaban in bulk and pharmaceutical dosage forms.

Keywords: Edoxaban, RP-HPLC, Quantitation, ICH guidelines, correlation coefficients, RSD %, accuracy

1 INTRODUCTION

Edoxaban is a direct, selective, reversible and competitive inhibitor of human factor Xa. In coagulation, uninhibited factor Xa forms a prothrombinase complex with factor Va on platelet surfaces. Prothrombinases turn prothrombins to thrombins. Thrombins turn blood-soluble fibrinogens to insoluble fibrins, which are the main components of blood clots[1]. Edoxaban is chemically known as N'-(5-chloropyridin-2-yl)-N-[(1S, 2R, 4S)-4-(dimethylcarbamoyl)-2-[(5-methyl-6, 7-dihydro-4H-[1, 3] thiazolo [5, 4-c] pyridine-2-carbonyl) amino] cyclohexyl] oxamide was shown in Figure 1. Literature review tells that very few analytical methods have been reported for the determination of Edoxaban which includes UV-Spectrophotometry [2]-[3], High performance liquid chromatography [4]-[10], and UPLC-Mass spectroscopy method [11-12]. The present study was aimed to develop a novel, simple, economic and validated RP-HPLC method for the estimation of Edoxaban according to ICH guidelines [13].

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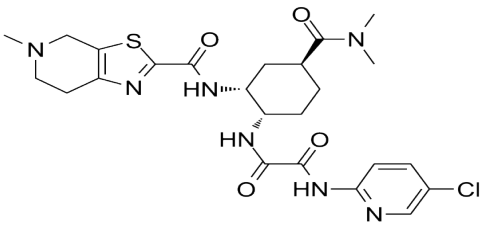

Figure 1: Chemical structure of Edoxaban

2 MATERIALS AND METHODS

2.1 Chemicals and Reagents

Edoxaban was procured from Hetero Drugs Ltd., Hyderabad, India. Acetonitrile of HPLC grade was procured from Merck Specialities Private Limited, Mumbai, India. Water and orthophosphoric acid of HPLC grade was obtained from Rankem Ltd., India. HPLC grade of Potassium dihdrogen phosphate buffer were procured from Rankem Ltd., India. Lixiana® tablets were procured from Daiichi-Sankyo Limited.

2.2 Instrumentation

An isocratic RP-HPLC method was performed on Shimadzu LC-20AT Prominence with SPD 20A detector HPLC system. Hypersil ODS C18 column with 100mm × 4.6 mm i.d. and 5 µm particle size column is used as a stationary phase. An ultrasonic bath sonicator (Frontline FS 4, Mumbai, India), semi-micro analytical balance (India) and Whatman filter paper No. 41 is used in the study.

2.3 Chromatographic conditions

In this work a reverse phase Hypersil ODS C18 column with 100mm × 4.6 mm i.d. and 5 µm particle size was chosen as stationary phase and mobile phase consisting of mixture of Potassium di-hydrogen phosphate (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v) was delivered at a flow rate of 1.0ml/min and detector wavelength at 230 nm. Injection volume was 20µl. The run time was 10min and the retention time of Edoxaban was found to be 3.677 min.

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2.4 Chromatographic Parameters

Equipment	:	Shimadzu LC-20AT Prominence HPLC system, equipped with SPD 20A detector
Column	:	Hypersil ODS C18 (100mm×4.6 mm, 5µm particle size)
Mobile Phase	:	Potassium di-hydrogen phosphate (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v)
Flow rate	:	1 mL/min
Detection Wavelength	:	230 nm
Injection volume	:	20 µL
Column temperature	:	Ambient
Run time	:	10 minutes

2.5 Preparation of mobile phase

2.5.1 Solution A Acetonirile HPLC-Grade

2.5.2 Solution B Accurately weighed about 6.8g of Potassium di-hydrogen phosphate (KH2PO4) was taken into 1000ml beaker and dissolved to 1000ml with HPLC grade water and degassed in ultrasonic water bath and filtered through 0.45µm filter using vacuum filtration and the pH was adjusted to 3.5 with orthophosphoric acid.

2.5.3 Mobile Phase Volume of Solution (A) and solution (B) taken in ratio 70:30 (v/v) and mixed well and filtered through 0.45µm membrane filter and degas for 10 minutes.

2.5.4 Preparation of diluent

Mobile phase was used as diluent.

2.5.5 Preparation of Edoxaban standard drug stock solutions

An accurately weighed quantity of Edoxaban 15mg was transferred to 100ml volumetric flask, dissolved in 100ml mobile phase, the final volume was made with mobile phase to obtain standard solution having concentration of 150µg/ml. These stock solutions were used to prepare further dilutions.

2.5.6 Preparation of Sample Solution

Sample solution was prepared from Lixiana® tablets. Twenty tablets of Lixiana® were taken and weighed individually and the average weight of twenty tablets was calculated. From this calculation the weight of each tablet is determined. Each tablet of Lixiana® tablets contains 15 mg of Edoxaban. After weighing, twenty tablets of Lixiana® were crushed and mixed in a mortar and pestle to produce powder. An accurately weighed

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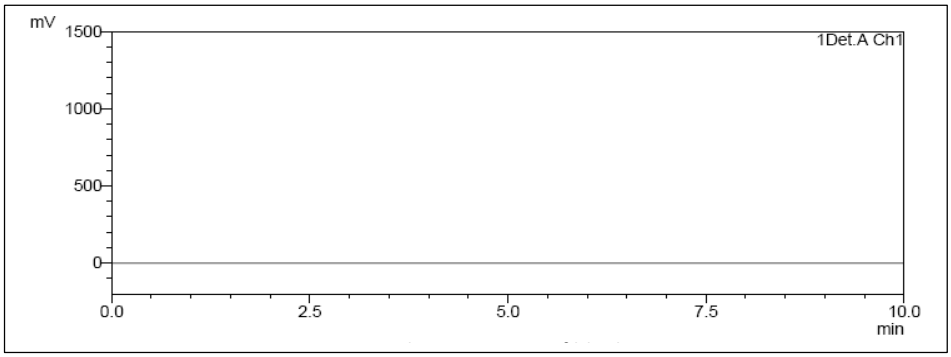
quantity of powder equivalent to 15 mg of Edoxaban were transferred into a clean and dry 100 mL volumetric flask and then mobile phase was added and sonicated to dissolve it completely and filtered through 0.45 µm nylon membrane filter and volume was made up to the mark with the same mobile phase to get the concentration of 150 µg/mL of Edoxaban. An aliquot of 10 mL was pipette out from the above solution and then transferred into a 100 mL of volumetric flask and diluted up to mark with the mobile phase to get the concentration of 15 µg/mL of Edoxaban solution.

2.5.7 Standard and sample solution for assay studies

An aliquot of 15 µg/mL of Edoxaban were injected six times into the chromatographic system and peak area for Edoxaban were measured and assay % was calculated by comparing the peak area of standard and sample chromatogram was shown in Table 1 and a typical chromatogram of blank, standard and sample solution of Edoxaban is shown in Figure 2, 3 and 4.



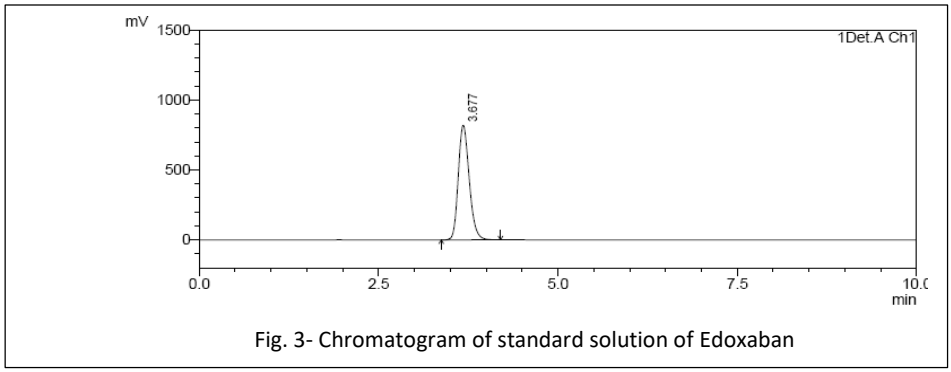


Fig. 3- Chromatogram of standard solution of Edoxaban

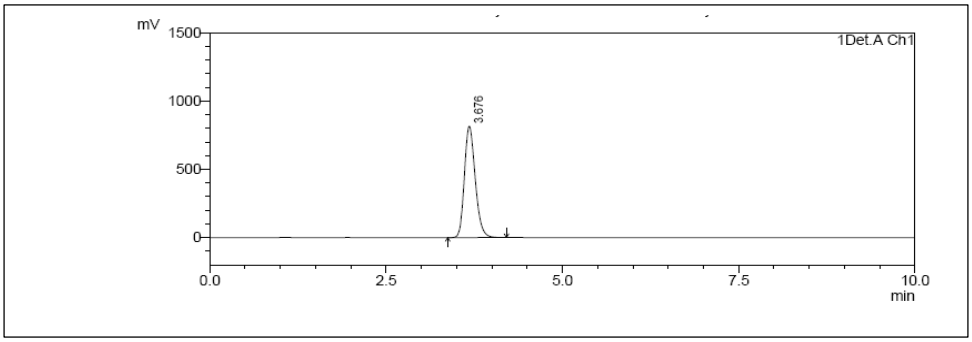


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Table 1: Results for assay studies

Drug	Lixiana® tablet Label claim (mg/tablet)	Amount found* (mg/tablet)	Label claim %	RSD %
Edoxaban	15	14.94	99.60	0.23

* Mean of six determinations

3 Optimization of RP-HPLC method

For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Potassium di-hydrogen phosphate (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v) using Shimadzu LC-20AT Prominence HPLC system, equipped with SPD 20A detector.

3. 1 System Suitability

Performance calculations were performed by calculating the system suitability parameters of the proposed RP-HPLC method for the estimation of Edoxaban in bulk and tablet dosage form. The results of system suitability parameters of the proposed RP-HPLC method for the estimation of Edoxaban in bulk and tablet dosage form are excellent and all results were complies within the acceptance limits and it confirms the system suitability of the proposed RP-HPLC method which was shown in Table 2.

Table 2: Performance calculations and system suitability parameters of Edoxaban

Parameters	Edoxaban	Acceptance limits
Retention time (min)	3.677	-----
Theoretical plates (N)	2677	Not less than 2000
Asymmetry factor	1.2	Not more than 2
Linearity range (µg/mL)	10.5-18	-----
Limit of detection (LOD) (µg/mL)	0.03	-----
Limit of quantification (LOQ) (µg/mL)	0.09	-----

3.2 Specificity

The effect of excipients and other additives usually present in the dosage form of Edoxaban in the determination under optimum conditions was investigated and confirms that there is no interference. The specificity of the RP-HPLC method was established by

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injecting the placebo solution into the HPLC system. The representative chromatogram of placebo was shown in Figure 5.

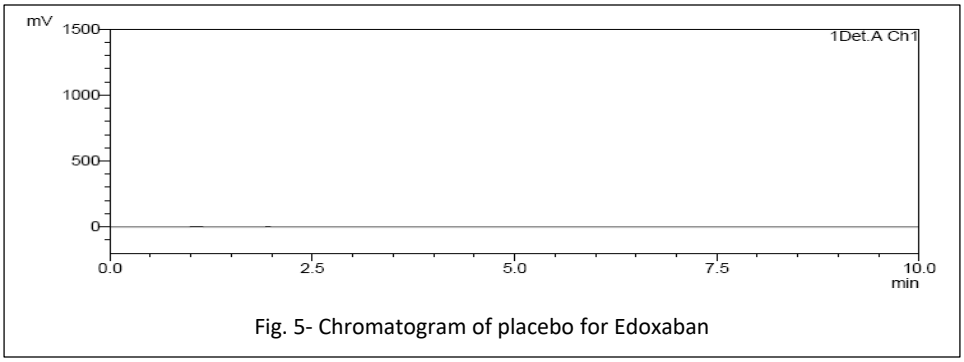

Fig. 5- Chromatogram of placebo for Edoxaban

3.3 Linearity

An aliquots of 0.7, 0.8, 0.9, 1, 1.1 and 1.2 mL from the standard drug stock solutions of 150 µg/mL of Edoxaban was pippetted out and transferred into the series of 10 mL of volumetric flask and volume make up to 10 mL with the mobile phase to get a concentration of 10.5, 12, 13.5, 15, 16.5 and 18 µg/mL of Edoxaban respectively. All the above solutions were filtered through 0.45 µm nylon membrane filter and then 20 µL of each solution was injected three times into the HPLC system. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. Calibration curve were constructed by plotting peak area versus concentration (µg/mL) is shown in Figure 6 and the regression equation were calculated and the results are presented in Table 3.

Table 3: Linearity of Edoxaban

Concentration of Edoxaban (µg/mL)	Peak Area
10.5	6711166
12	7646139
13.5	8520297
15	9648784
16.5	10511273
18	11530731

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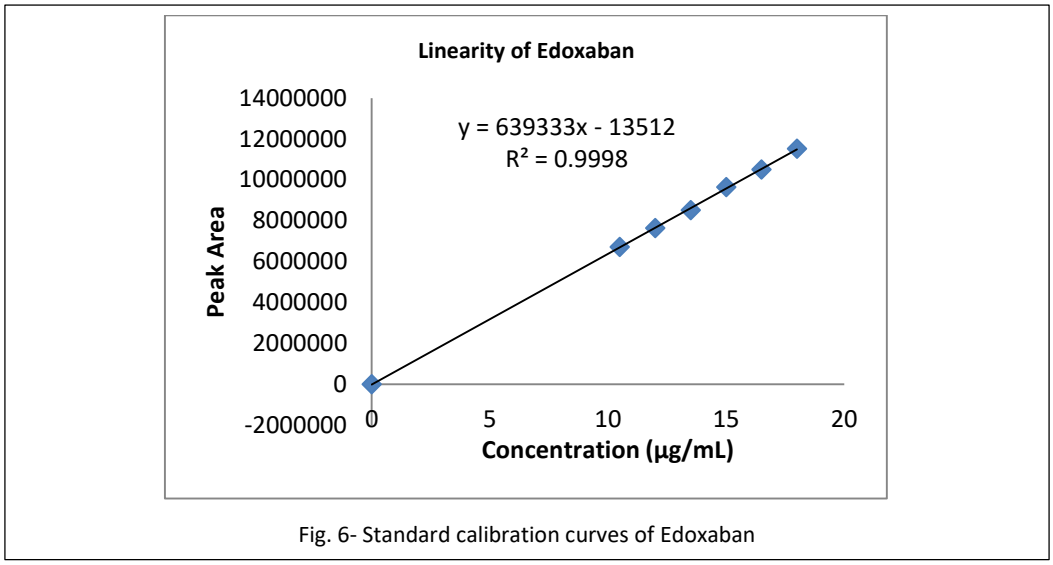

Fig. 6- Standard calibration curves of Edoxaban

3.4 Accuracy studies

The accuracy of the proposed method was determined by calculating the recovery of Edoxaban by standard addition method. Recovery studies were carried out by adding concentration level of 80 %, 100 % and 120 % of standard drug solution of Edoxaban to the pre-analysed sample solution of Lixiana® tablet powder and the mixtures were re-analysed by the proposed method. Three replicates were prepared for each concentration level and was injected into the HPLC system and the results were obtained by using following formula and also confirm the accuracy of the proposed method were reported in Table 4.

Table 4: Results of accuracy studies of Edoxaban

Concentration Level in %	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery	RSD %
S_1:80%	12	11.98	99.83		
S_2:80%	12	11.97	99.73	99.74	0.09
S_3:80%	12	11.96	99.65		
S_4:100%	15	15.10	100.67		
S_5:100%	15	15.11	100.71	100.70	0.03
S_6:100%	15	15.11	100.72		
S_7:120%	18	17.99	99.98		
S_8:120%	18	18.06	100.35	100.27	0.25
S_9 :120%	18	18.08	100.47		

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3.5 Precision studies for Edoxaban

The precision of the proposed method was performed to express the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the optimized conditions. Precision was performed by injecting six times of a homogenous sample preparation of 15 µg/mL of Edoxaban into the HPLC system to ensure that the analytical method is working properly. The results of precision of Edoxaban were reported in Table 5. The intermediate precision (also known as Ruggedness) of the method was evaluated by performing precision on within-laboratories variations such as different days with different analysts. Intermediate precision was carried out by injecting six times of a homogenous sample preparation of 15 µg/mL of Edoxaban into the HPLC system on different days with different analysts to ensure that the analytical method is rugged. The results of intermediate precision or ruggedness of Edoxaban were reported in Table 6.

Table 5: Precision of Edoxaban

Concentration (µg/mL)	Peak Area
15	9574871
15	9593898
15	9580037
15	9567126
15	9577657
15	9597188
Average	9581796
SD	11547.831
RSD %	0.121

Table 6: Ruggedness of Edoxaban

Concentration (µg/mL)	Day-1 Analyst-1 Peak Area	Day-1 Analyst-1 Peak Area
15	9703031	9743353
15	9707077	9770866
15	9709260	9773762
15	9706456	9762660
15	9777657	9593898
15	9797188	9580037
Average	9733445	9704096
SD	42311.876	91451.269
RSD %	0.43	0.94

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3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection is a smallest concentration of an analyte which gives a measurable response. Limit of quantitation is a smallest concentration of an analyte that gives a measurable response which can be quantified accurately. LOD and LOQ are calculated by using following formula and the results of LOD and LOQ of Edoxaban were reported in Table 2.

$$\text{Limit of Detection (LOD)} = 3.3\times \frac{\text{Standard deviation of the response}}{\text{Slope of the calibration curve}}$$

$$\text{Limit of Quantitation (LOQ)} = 10\times \frac{\text{Standard deviation of the response}}{\text{Slope of the calibration curve}}$$

3.7 Robustness

Robustness of the method was carried out by deliberately changing the mobile phase composition by altering the proportion of organic phase by ±10 % and flow rate by ±0.1 mL. There are no marked variations were observed in the system suitability parameters and the results of robustness were reported in Table 7 ensures that the developed analytical method remain unaffected by a small or deliberate changes in chromatographic method parameters and gives an indication of its reliability during normal usage.

Table 7: Robustness data of Edoxaban

Variations in method parameters	Retention Time (mins)	Average peak area*	RSD %	System suitability parameters Theoretical Plates	Asymmetry
Buffer: Acetonitrile (37:63, v/v)	3.683	9567126	0.16	2727	1.2
Buffer: Acetonitrile (23:77, v/v)	3.677	9577657	0.71	2744	1.2
0.9 mL/min			0.63	2720	1.2
Flow rate	3.679	9597188			
1.1 mL/min			0.32	2717	1.2
Flow rate	3.682	9580037			

* Mean of six determinations

4 RESULTS AND DISCUSSION

The present RP-HPLC method for the estimation of Edoxaban in bulk and pharmaceutical dosage forms was established and validated as per ICH guidelines. This method was intended for rapid and accurate estimation of Edoxaban in bulk and pharmaceutical

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dosage forms. Good separation of the chromatographic peaks was observed and no interfering peaks are found. A number of commercially available HPLC columns and various mobile phases were used for the development of RP-HPLC method for estimation of Edoxaban in bulk and pharmaceutical dosage forms. The best response was obtained with Hypersil ODS C18 (100mm×4.6 mm, 5µm particle size); Shimadzu LC-20AT Prominence HPLC system, equipped with SPD 20A detector and mobile phase contained a mixture of Potassium di-hydrogen phosphate (p^H adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v) was delivered at a flow rate of 1 mL/min. Quantitation was attained at 230 nm depends on peak area. The retention time of Edoxaban was 3.677 min. Linearity was established for Edoxaban in the range of 10.5-18 µg/mL with correlation coefficients (r=0.999) and the percentage recoveries were between 99.74 %-100.70 % for Edoxaban respectively. The RSD % values of accuracy for Edoxaban were found to be < 2 %, which indicate accuracy of the proposed method. The RSD % values of precision were found to be 0.121% for Edoxaban respectively and for ruggedness were found to be 0.43% and 0.94 % for Edoxaban respectively, reveal that the proposed method is precise. LOD values were found to be 0.03 µg/mL for Edoxaban and LOQ values were found to be 0.09 µg/mL for Edoxaban. The RSD % values of robustness studies were found to be < 2 %, which indicate robustness of the proposed method. These reports show that the proposed method was accurate and precise for determination of Edoxaban in bulk and pharmaceutical combined dosage forms. The developed method is simple, precise, and accurate. Hence, the RP-HPLC method can be applicable for the routine analysis of Edoxaban in bulk and pharmaceutical dosage forms.

5 CONCLUSIONS

RP-HPLC method for the estimation of Edoxaban in their bulk and pharmaceutical dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Edoxaban in the range of 10.5-18 µg/mL with correlation coefficient 0.999. The percentage recovery of drug was achieved in the range of 98-102% which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Edoxaban in their bulk and pharmaceutical dosage form.

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