FORMULATION AND EVALUATION OF PH-DEPENDENT COLON-TARGETED TABLETS OF RIFAXIMIN BY DESIGN OF EXPERIMENT

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ABSTRACT

Objective: The research is designed at formulating and evaluating pH-sensitive rifaximin colon-targeted tablets for targeted action in proximal colon.

Method: The colon-targeted tablets are done by granulation of three levels of polymers such as Eudragit L30D, Carbopol 974P, and ethyl cellulose. The evaluation parameters such as swelling studies, drug dissolution, *in vitro* drug release studies, stability, and the Fourier transform infrared studies carried out for optimized formulations.

Results: Physicochemical parameters of all the 27 formulations (RF1-RF27) evaluated and RF21 is chosen for further investigation based on weight variation, hardness, drug content, and swelling index. The *in vitro* drug release studies indicate that the optimized formulation RF21 released 98.75% drug within 24 h. The stability studies indicate that the formulation is stable.

Conclusion: An effective and stable pH-dependent rifaximin colon-targeted tablet formulated for the targeted treatment of bowel syndrome.

Keywords: Rifaximin, Colon drug delivery system, Swelling index, Irritable bowel syndrome.

INTRODUCTION

The drug delivery systems are aimed at supplying the drug to an object site in a body. Drug delivery systems alter drug dissolution profile for increasing the product efficacy [1]. Colon drug delivery system is targeted to deliver the drug to of gastrointestinal (GI) tract [2]. Conventional oral administrated drugs lack the special property of drug targeting to GI sites. The colon-targeted drug delivery systems are applied for the treatment of bowel diseases and colonic cancer. The colon-specific drug delivery systems (CDDSs) protect the drug till it reaches the colon, ensuring that the drug is not released in stomach or small intestine. They possess long retention time of 4–5 days, thus facilitating maximum adsorption of drug, hence, making it ideal for targeted delivery [3]. CDDS applied to reduce the dosing frequency of drug, to deliver the drug to regions that have less hostile metabolic, to increase the pharmacological activity, and to prevent drug from degradation [4].

CDDS depends on pH and spreadability of the drug, the delivery system, GI transits time and the interaction level between the same [5]. The release of drug in GI tract also depends on ability of enzymes to break bonds between drug and carrier molecules. CDDSs with maximum drug release are considered ideal for colon release.

Among various approaches, the less suitable method is pH-dependent system due to variations in pH of GI tract. In spite of these intra- and inter-subject variations, these were considered better when combined with time-dependent systems [6].

Rifaximin is used in the treatment of hepatic encephalopathy, diarrhea, and bowel syndromes. Rifaximin acts by inhibiting RNA synthesis in susceptible bacteria by binding to the beta-subunit of bacterial deoxyribonucleic acid-dependent ribonucleic acid polymerase enzyme [7]. This blocks translocation steps that form phosphodiester bond. Rifaximin is selected as colon tablet due to its poor oral availability.

EXPERIMENTATION

Materials

Rifaximin was procured from Hetero Drugs Ltd., Hyderabad, India. Eudragit RL 100, HPMC K4M, and ethyl cellulose were purchased from Aurobindo Labs, India. All other chemicals and solvents were used without further purification.

Instruments

The formulations carried out using tablet compression machine (Cadmach, Ahmedabad) and dissolution apparatus (Electrolab [TDT- 08L], Mumbai). Chemical analysis was performed using Fourier transform infrared (FTIR) spectrophotometer (Shimadzu 8400S, Japan) and ultraviolet (UV)-visible spectrometer (Shimadzu, Japan).

Preparation of colon-targeted tablets of rifaximin

Twenty-seven formulations (RF1-RF27) were prepared using polymers such as Eudragit L30D, Carbopol 974P, and ethyl cellulose. The 3^3 response surface method adopted for design of experiment. The formulations with varied polymer concentration prepared. All the prepared formulations were constituted with magnesium stearate. The mixture was passed through sieve no 85. The polyvinylpyrrolidone K 30 and 5% in isopropyl alcohol were chosen as binder solvent for granulation. The wet crude passed through sieve no 12#. Contents dried for 2 h at 45°C. The obtained material was compressed with 10 mm flat punch after screening through sieve no.18#.

Evaluation of rifaximin colon-targeted tablets

Weight variation test

The average weight of 20 tablets determined randomly. Each tablet weighed individual and checked for deviation from average weight. The percentage deviation tabulated [8].

Thicknesses

The thickness measured in millimeters using a screw gauge for 10 tablets. The average thickness calculated. The deviation of each of 10 tablets from average thickness noted as the standard deviation.

Hardness

The hardness is measured as tensile strength in kg/cm^2 by tablet hardness testing machine. Randomly selected three tablets and tested for hardness. The average of all three values calculated [9].

Friability

The Roche friabilator (Electrolab, India) is used for determining friability of tablets. Twenty tablets placed in machine and run for 100 revolutions. The tablets weighed after dusting [10].

The friability (F %) is calculated by the formula,

$$F\% = (1 - W_0/W) \times 100.$$

Drug content

The average weight of 20 tablets calculated. 10 mg of tablet powder mixed with 100 ml of phosphate buffer (pH 7.2) for complete dissolution. Contents filtered and filtrate checked for drug concentration at 227 nm by UV spectrometer.

In vitro swelling index

The pre-weighed tablet placed in 5 ml of phosphate buffer (pH 7.2) for 12 h tablet weight at intervals of 1, 2, 4, 8, 10, and 12 h by taking it out from solvent using a filter paper [10]. The swelling index obtained using the following formula.

$$\text{Swelling index (S.I)} = (Wt - Wo)/Wo \times 100,$$

Where, S.I=Swelling index,
Wt=Weight of tablet time t,
Wo=Initial weight.

In vitro drug release study

The dissolution of prepared tablet formulations was carried out by obeying various conditions. The dissolution medium chosen is combination of acidic buffer pH 1.2 (2 H), phosphate buffer pH 6.8 (4 H), and phosphate buffer (pH 7.2) in 900 ml dissolution medium. The drug content estimated at intervals of 1, 2, 3, 4, 6, 8, 10, 12, and 24 h by UV spectroscopy at 227 nm [11].

Drug release kinetics

The drug release data fitted into various linear and non-linear models and checked for linearity [12].

Table 1: Evaluation tests of rifaximin colon-targeted tablets

F. No.	*Weight variation (mg)	#Thickness (mm)	#Hardness (kg/cm^2)	#Friability (%)	#Drug content (%)	Swelling index (%)
RF1	801.65±1.2	6.0±0.12	6.5±0.12	0.53±0.01	96.23±0.63	75±0.76
RF2	798.69±0.8	6.2±0.06	6.4±0.06	0.56±0.02	96.04±0.06	78±0.72
RF3	798.04±0.5	6.0±0.06	6.3±0.06	0.61±0.03	97.56±0.14	79±0.64
RF4	801.05±0.0	6.2±0.12	6.1±0.12	0.71±0.01	97.11±1.01	87±0.81
RF5	801.54±0.4	6.3±0.00	6.2±0.00	0.64±0.02	95.23±0.8	74±1.03
RF6	800.78±0.4	6.3±0.10	6.8±0.06	0.65±0.01	96.45±0.31	75±0.84
RF7	800.65±0.3	6.2±0.10	6.4±0.10	0.57±0.02	95.18±0.49	72±0.72
RF8	799.57±0.2	6.2±0.25	6.2±0.40	0.68±0.01	96.23±0.51	83±0.79
RF9	800.76±0.35	6.1±0.06	6.5±0.06	0.59±0.00	97.13±0.56	83±0.80
RF10	800.49±0.2	6.3±0.20	6.1±0.42	0.78±0.02	96.23±0.24	78±0.46
RF11	801.53±0.4	6.1±0.06	6.3±0.06	0.75±0.01	98.97±0.21	87±0.67
RF12	802.58±0.3	6.1±0.00	6.5±0.06	0.74±0.02	96.45±0.76	89±0.93
RF13	801.34±0.2	6.2±0.26	6.7±0.35	0.73±0.02	98.45±0.48	97±0.53
RF14	798.67±0.3	6.2±0.21	6.5±0.21	0.75±0.03	97.98±0.23	84±1.08
RF15	799.65±0.2	6.3±0.06	6.8±0.23	0.76±0.02	96.45±0.36	91±0.91
RF16	800.65±0.3	6.1±0.25	6.5±0.23	0.76±0.01	95.45±0.69	87±0.63
RF17	801.79±0.4	6.4±0.15	6.7±0.32	0.77±0.01	94.34±0.35	94±0.48
RF18	801.87±0.1	6.4±0.25	6.6±0.35	0.81±0.01	96.56±0.23	96±0.90
RF19	799.67±0.3	6.2±0.12	6.3±0.12	0.80±0.03	97.29±0.34	91±0.75
RF20	799.32±0.2	6.3±0.12	6.4±0.2	0.65±0.03	98.18±0.81	89±0.67
RF21	800.27±0.4	6.2±0.06	6.3±0.06	0.61±0.02	99.48±0.69	98±0.54
RF22	800.27±0.1	6.3±0.12	6.2±0.12	0.52±0.03	97.18±0.07	97±0.67
RF23	800.26±0.13	6.1±0.17	6.7±0.4	0.77±0.05	98.14±0.76	86±0.54
RF24	800.10±0.5	6.2±0.00	6.8±0.23	0.72±0.08	98.16±0.12	94±0.86
RF25	799.12±0.6	6.2±0.17	6.7±0.12	0.66±0.02	97.23±0.00	92±0.70
RF26	800.16±0.8	6.3±0.10	6.5±0.21	0.71±0.89	97.78±0.23	93±0.68
RF27	800.29±0.15	6.4±0.29	6.4±0.45	0.86±0.03	98.10±0.40	95±0.75

*Values are expressed in mean±standard deviation: (n=20), #Values are expressed in mean±standard deviation: (n=3)

Table 2: Release kinetics of optimized formulation of rifaximin colon-targeted tablets (RF21)

Formulation code	Zero order		First order		Higuchi		Korsmeyer–Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
RF21	0.996	4.165	0.939	0.087	0.937	23.24	0.929	0.865

Table 3: Release kinetics of marketed product

Formulation code	Zero order		First order		Higuchi		Korsmeyer–Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
Marketed formulation	0.936	4.196	0.891	0.083	0.926	19.15	0.909	0.747

Stability study

The stability of drug tested at 40°C±2°C/75% RH±5% RH for 3 months using stability chamber. Sample stability checked at 0, 30, 60, and 90 days period according to ICH guidelines [13].

RESULTS AND DISCUSSION

The preliminary drug authentication was carried out by UV spectrophotometry. The pure rifaximin exhibited absorption maxima at 227 nm. Standard calibration curves of the drug plotted in acidic buffers of pH 1.2, 6.8, and 7.2 to check the stability of drug in respective condition. The results show that the graphs are linear over 2–12 µg/ml with R^2=0.999 indicating stability of the drug.

Initially, 27 formulations of rifaximin (RF1-RF27) were prepared by wet granulation using three variables and three levels of polymers of different Eudragit L30D, Carbopol 974P, and ethyl cellulose (low, middle, and high concentrations) by design of experiment.

Evaluation parameters of rifaximin colon-targeted tablets

The rifaximin colon-targeted tablets were prepared by varying proportions of polymers by wet granulation method and further evaluated for following evaluation tests. The results are found to be within the pharmacopoeia limits (Table 1).

The weight variations of all formulations are in the limit allowed that is ±5% of total tablet weight. The crushing strength of fabricated tablets is between 6.0 and 7.0 kg/cm². The thickness of all the formulations is between the ranges of 6 and 6.4 mm. The friability was found to be between 0.52 and 0.8%. The drug content of all formulations is in between 95.18 and 99.48%, indicating even distribution of drug in all the formulations. The swelling index of the tablet increases with increase in time up to 12 h indicating the drug remains in intestinal region until the drug is released completely from the delivery system and promotes evacuation after its release.

***In vitro* drug release studies**

The release of rifaximin from different formulations is recorded in Figs. 1-4. The studies extended up to 24 h. The highest drug release of 98.75% found in RF21 of within 24 h. Hence, RF21 is chosen for further investigations. The drug release of the marketed product is 82.23% up to 24 h. A comparative *in vitro* study plot of optimized formulation (RF21) and marketed tablet (conventional and sustained) was recorded (Fig. 5).

***In vitro* drug release kinetics for optimized (RF21) formulation and marketed product**

Drug release kinetics was evaluated for optimized formula of rifaximin colon-targeted tablets (RF21) and marketed formula (Table 2). The regression coefficient of 0.996 for zero-order plot, i.e., indicates zero-order mechanism for the drug release. Further, n value obtained from the Korsmeyer–Peppas plots, i.e., 0.865 indicating non-Fickian (anomalous) transport. The marketed conventional formulation also followed the zero-order kinetics (Table 3).

Design of experiment

This method is mainly used to explain the effect of each factor on other factors, whether this effect is significant or not, if significant how it influences the response. In this present work, the effect of ethyl cellulose on other two Eudragit L30D and Carbopol 974P is explained.
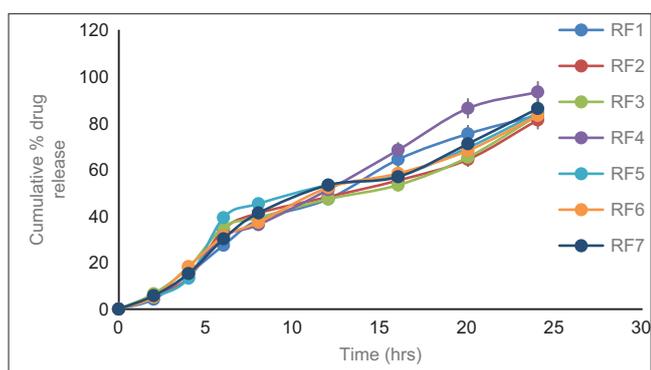
Fig. 1: *In vitro* drug release profile for rifaximin colon-targeted tablets RF1-RF7
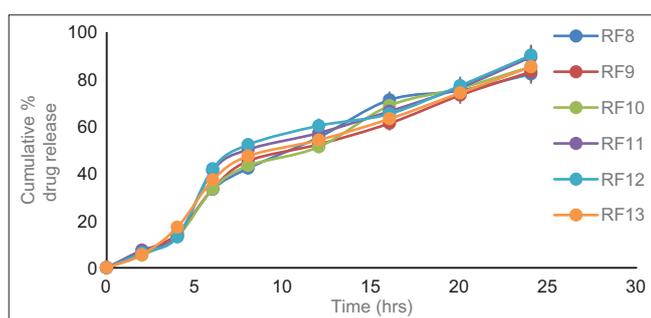
Fig. 2: *In vitro* drug release profile for rifaximin colon-targeted tablets RF8-RF13
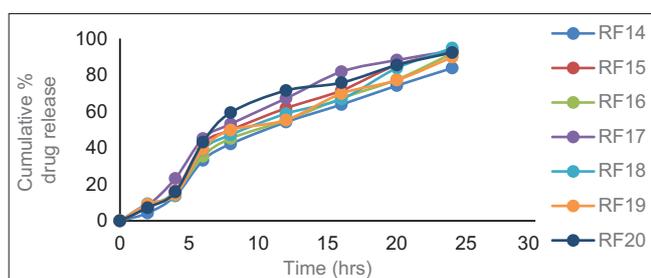
Fig. 3: *In vitro* drug release profile for rifaximin colon-targeted tablets RF14-RF20
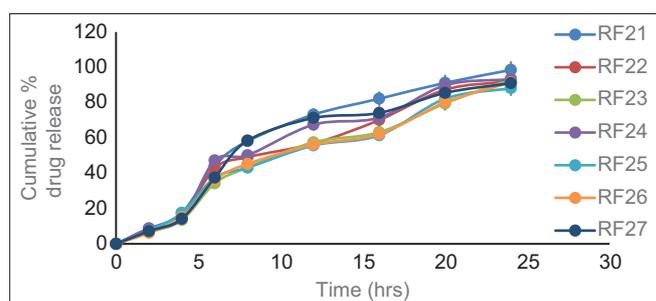

Fig. 4: *In vitro* drug release profile for rifaximin colon-targeted tablets RF21-RF27
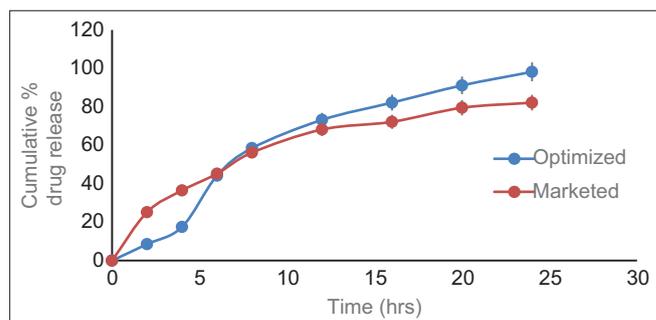

Fig. 5: Comparative *In vitro* study plot of rifaximin colon-targeted tablets optimized formulation (RF21) and conventional marketed tablet

Table 4: Parameters after accelerated stability study of rifaximin colon-targeted tablet formulation (RF21)

Parameters	Temperature maintained at 40±2°C; RH maintained at 75±5%RH			
	Initial	After 1 month	After 2 months	After 3 months
Drug content (%)	99.48±0.69	98.46±0.65	98.33±0.37	98.32±0.22
In vitro drug release (%)	99.48±0.69	99.39±0.53	99.25±0.42	97.22±0.35
Swelling index (%)	98±0.54	98.0±0.46	98.0±0.37	98.0±0.23
Hardness (Kg/cm^2)	6.3±0.66	6.3±0.58	6.3±0.45	6.3±0.37

RH: Relative humidity
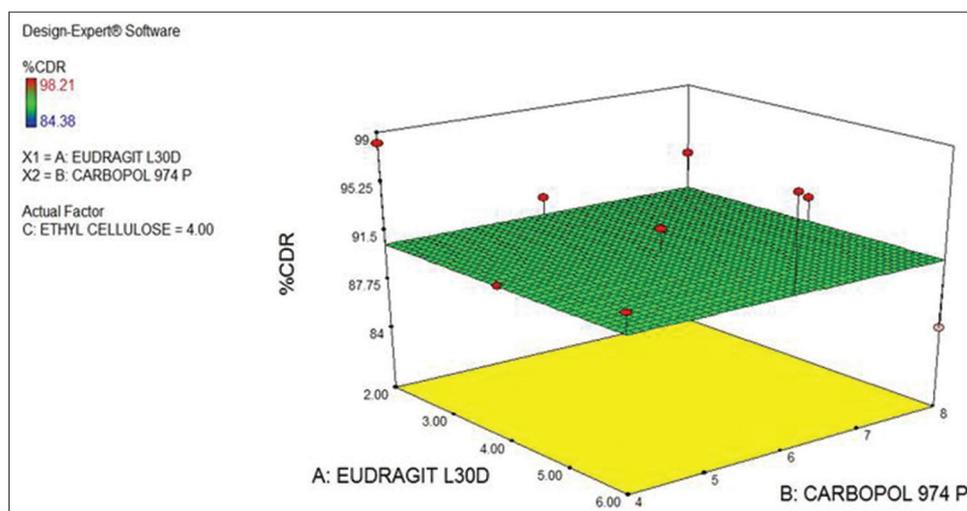
Fig. 6: Response surface plot showing the influence of amount of polymer on the release profile of rifaximin colon tablets for cumulative percentage drug released
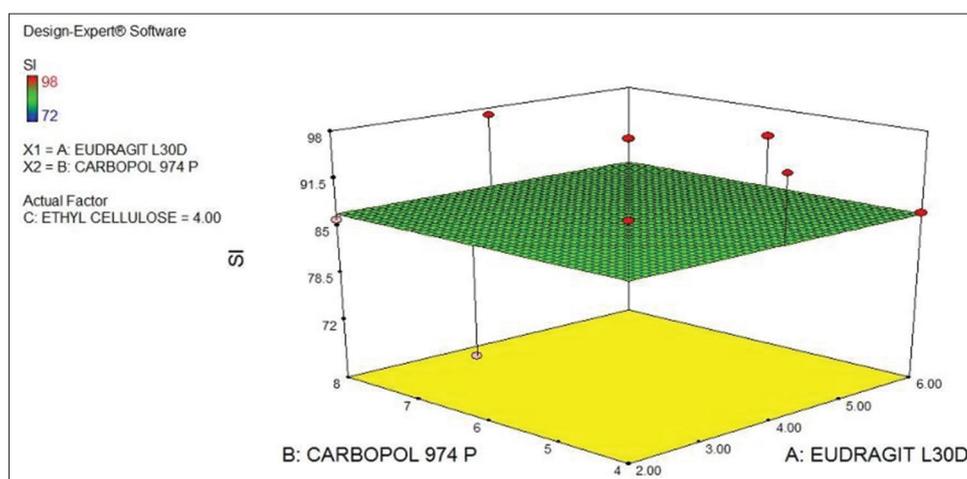

Fig. 7: Response surface plot showing the influence of amount of polymer on swelling index of rifaximin

In Fig. 6, the effect of ethyl cellulose on percentage cumulative drug release shows significant effect of ethyl cellulose on percentage cumulative drug release. The percentage cumulative drug release of all the three formulations is in between 84.38 and 98.21% but when ethyl cellulose is in low concentration, the maximum percentage cooldown reduction is near 84.38%. This is the effect of factor (ethyl cellulose) on response which is a negligible on swelling index as formulations exhibit excellent swelling property and there is slightly influence on swelling index by ethyl cellulose (Fig. 7). Overlay plot of rifaximin colon-targeted tablets is represented in Fig. 8.

Drug-excipient compatibility studies
FTIR studies spectroscopy
The FTIR spectrum of rifaximin and optimized formulation is shown in Figs. 9 and 10. The O-H and C=O stretching in rifaximin pure drug were observed at 3448 and 1745 cm^{-1}, respectively, which remained intact in physical mixture of rifaximin and excipients. C-H stretching of pure drug is at 2924 cm^{-1}, whereas it shows the same peak for physical mixture also. C-H bending of pure drug and physical mixture is at 790 cm^{-1}. The FTIR spectrum of rifaximin optimized formulation RF21 also exhibited characteristic bands (3437 and 1786 cm^{-1} for O-H and C=O stretching and 2924 cm^{-1} for C-H stretching, and 790 cm^{-1} for C-H bending) consistent with the molecular structure of rifaximin pure drug which indicated that no chemical interaction occurred between the drug and excipients used in the formulation.

Stability studies
The stability studies of the optimized formulation (RF21) indicated no significant effect on drug release, hardness, drug content, and swelling study data. Hence, the formulations are stable (Table 4).
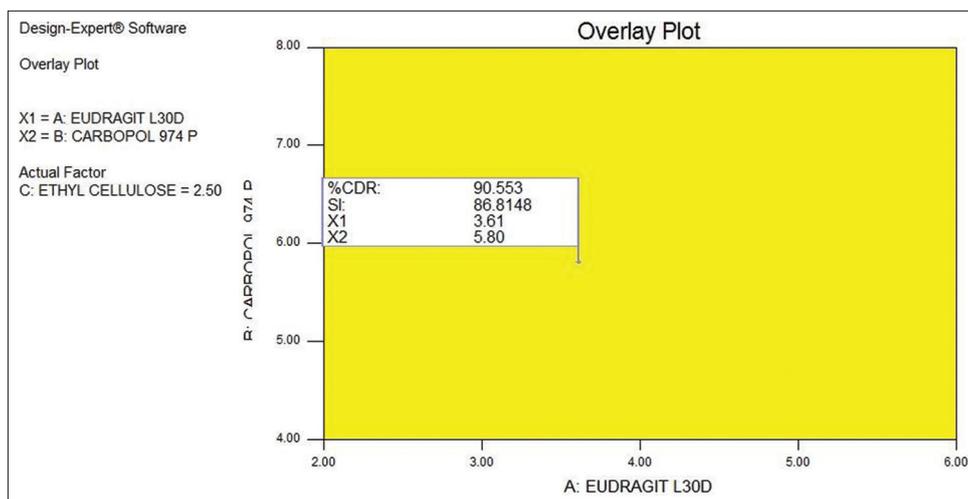
**Fig. 8: Overlay plot of rifaximin colon-targeted tablets**
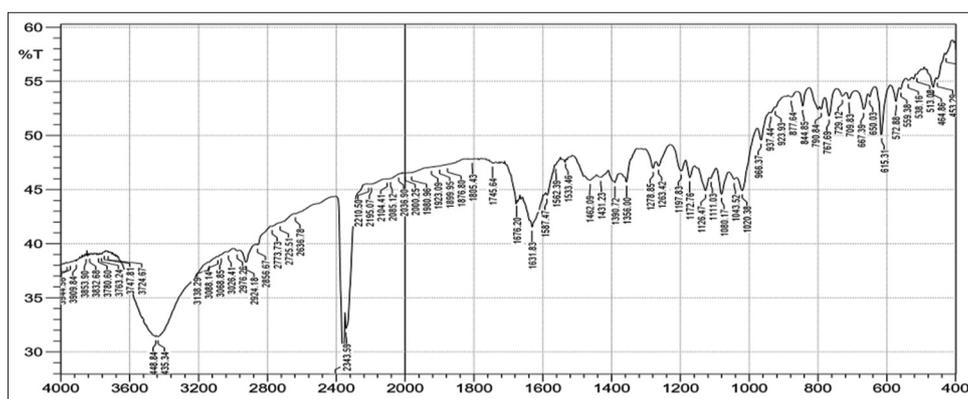
Fig. 9: Fourier transform infrared spectrum of pure drug rifaximin
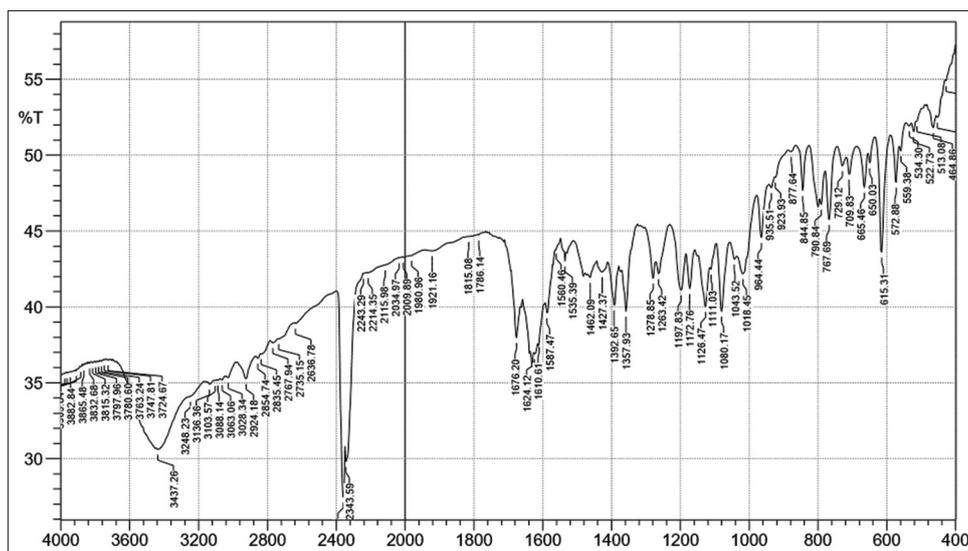

Fig. 10: Fourier transform infrared spectrum of optimized formulation of rifaximin colon-targeted tablets

CONCLUSION

The present study is conducted to formulate pH-sensitive matrix tablets of rifaximin for targeted action in proximal colon. Twenty-seven formulations of rifaximin (RF1-RF27) were prepared using three levels of polymers of different Eudragit L30D, Carbopol 974P, and ethyl cellulose (low, middle, and high concentrations) by design of experiments software. The results of physicochemical parameter test applied on all the formulations designated that the weight variations are within allowed limit. The drug content measured for formulations indicates uniform drug distribution. Swelling index indicates that drug will remain in intestinal region until the drug is released completely from the delivery system and promotes evacuation after its release. Drug compatibility studies carried out by recording the FTIR spectra indicate no chemical interaction between the drug and the excipients used. *In vitro* drug

release of colon-targeted rifaximin tablets studied up to 24 h. The highest drug release of 98.75% found in the RF21 at within 24 h. Drug release kinetics for optimized formulation RF21 indicates that it follows zero-order mechanism. The stability studies indicate no major changes in swelling index, hardness, drug content, and *in vitro* drug release.

CONFLICTS OF INTEREST

No conflicts of interest by authors.

AUTHORS' CONTRIBUTIONS

All authors contributed equally.

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