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Original Article

Bio-analytical Method Development for Pharmacokinetic Study of Novel Lafutidine Formulation in Rabbit Plasma Using RP-HPLC

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ABSTRACT

Objectives: The main objective of the study is to carry out comparative *in vivo* pharmacokinetic study of novel formulation with marketed formulation of lafutidine using HPLC method. **Materials and Methods:** The animals were selected according to the study protocol and divided in to three groups. The animals were given lafutidine marketed and novel formulation orally; at predetermined time blood samples were collected and subjected to the protein precipitation to collect blood plasma. The plasma samples were analysed using validated RP- HPLC method. Analysis of plasma was done at 215 nm using Hypersil silica, C_{18} 250×4.6 mm. 5µ 0.02 M phosphate buffer and acetonitrile at 30:70 v/v was selected as mobile phase and passed at flow rate of 1.0 mL/min. **Results:** The established method was specific and sensitive for plasma sample. Calibration curve was developed using concentration range of 50-200 ng/mL with correlation coefficient 0.9993. The accuracy the intra and inter day precision was found 100.26 and 98.02% with percent accuracy 98.41% to 100.77% and 98.23% and 99.67%. **Conclusion:** The developed method is able to meet all specifications and reproducible to identify and quantify the lafutidine in rabbit plasma and can applied to study pharmacokinetic parameters.

Keywords: *In vivo* study, Pharmacokinetic, Novel formulation, Rabbit plasma, HPLC.

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INTRODUCTION

Lafutidine is a second-generation histamine H2 receptor antagonist that prevents gastric secretion[1,2] by multimodal mechanism. Unlike other antagonists, lafutidine prevents postprandial gastric acid secretion.[3,4] Lafutidine exerts anti-secretory action by blocking the H2 receptor. It induces collagen synthesis, has cytoprotective activity on gastric mucosa and restores damaged mucosa by increasing mucus biosynthesis. Lafutidine increases the Ca^{2+} ion concentration by increasing CGRP, which suppresses acid by decreasing gastrin, thus decreasing vagal tone. It also increases plasma somatostatin levels, which decrease gastrin release from G-cells. It blocks the attachment of gram-negative H. pylori to gastric mucosal cells.[5] The structure of lafutidine is shown Figure 1.

Lafutidine belongs to class II of BCS with a log P value of 3.8. The drug has a pKa value of 3.9. It is selectively absorbed from the upper part of the small intestine[1,7] with a biological half-life of 1-2 hr.[6,8] Lafutidine is used in the treatment of heart burns, gastric ulcers, duodenal ulcers, gastritis and Gastro-Oesophageal Reflux Disease (GORD).[1,7,9] The drug is also used in the treatment of dyspepsia. The drug has poor bioavailability due to its short half-life and selective absorption, which make it appropriate drug candidate for gastroretentive raft system. Many stability indicating methods have been developed for the estimation of lafutidine in bulk formulations, such as HPTLC,[10] RP-HPLC,[11] UPLC,[12] and UV spectroscopy.[13] Lafutidine estimation in human plasma, such as LC-ESI-MS,[14] and HPLC-MS[15] has been reported. In the present study, an effort was made to compare the *in vivo* pharmacokinetics of lafutidine in a marketed formulation and a novel raft formulation in rabbit plasma using validated RP-HPLC. The study received approval from the institutional ethical committee, Centralized Experimental Animal Division of Shadan Institute of Medical Sciences Hyderabad. The Protocol Approval No: IAEC-03/SES/2020/003.

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MATERIALS AND METHODS

Materials

Lafutidine was obtained as a gift sample from Sun Pharmaceutical Gujarat, India. Lafutidine conventional tablets were obtained from a local chemist shop. Methanol, acetonitrile, ortho-phosphoric

acid water and all other chemicals of HPLC grade were procured from Merck, Mumbai, India.

Formulation preparation and optimization

Raft forming gastroretentive drug delivery system are the preparation which are solution form in the container but when ingested orally due to change in pH due acidic environment of stomach undergo gelation which results in cohesive gel which floats on gastric content. In the present research Lafutidine raft systems are prepared by blending the drug with selected polymer namely sodium alginate, HPLCK4M and xanthan gum. Design of experiment approach was used to optimize the formula. A special three factor three level Box Behnken Design (BBD)[16] opted for optimization which generated 17 experimental runs. By fixing the concentration of drug (200 mg/ 10 mL), sodium bicarbonate (0.5% w/v), $CaCO_3$ (2.5% w/v) and $CaCl_2$ (0.001% w/v), the variables were evaluated at 3 different levels of low (-1), medium (0), and high (+1). On the basis of preliminary studies, the factors like amount of sodium alginate (0.5 to 1.5% w/v), amount of HPMC K4M (1 to 2% w/v) and amount of xanthan gum (0.5 to 1% w/v) were identified as formulation variables and dependent variables are buoyancy time, percent drug release at 1 hr, and percent drug release at the end of 12 hr.

The response of these variables in experimental domain was evaluated by Stat-Ease Design Expert® software V8.0.1 by applying one-way ANOVA at 0.05 levels.

For optimization each parameter was analysed using the F test and the quadratic model of the form was generated using Multiple Linear Regression Analysis (MLRA), where

$$Y=\beta+\beta1X1+\beta2X2+\beta3X1X2+\beta4X12+\beta5X22$$

"*Y* is the level of the measured response; *β* is the intercept *β1 to β2* are the regression coefficients. *X1 and X2* stand for the main effects; *X1X2* is the interaction between the main effects; *X12* and *X22* are the quadratic terms of the independent variables that were used to simulate the curvature of the designed sample space".

The formulation was prepared according to the procedure[17] mentioned in the reference and evaluated.

Evaluation of raft novel formulation

The optimized formulation was subjected to various evaluation studies such as physical appearance, pH, *in vitro* gelation, density, viscosity, raft strength, raft resilience, acid neutralization capacity *in vitro* release study drug polymer compatibility studies and stability studies according to reference procedure given.[18]

In vivo pharmacokinetic study of formulation

The study was conducted to evaluate pharmacokinetic parameters such as C_{max}, T_{max}, Ke, AUC and MRT in rabbit plasma using the RP-HPLC method.

Details of animals and research center

Name of animal: Rabbit.

Breed: New Zealand white rabbit.

Body weight: 2-3 kg.

Age: 5-6 months.

Breeding center: Animal House of Shadan Institute of Medical Sciences. Hyderabad, Telangana.

Ethical approval

To conduct the animal study, the study protocol was approved by the institutional ethical committee, of the Centralized Experimental Animal Division of Shadan Institute of Medical Sciences Hyderabad. The Protocol Approval No: IAEC-03/SES/2020/003.

Animal selection and study design

To carry out the study, 18 healthy (New Zealand white) rabbits of either sex were selected. For the study animals were divided into three groups of 6 animals each, A, B and C. Group A received marketed formulations, and B group received novel formulation and group C served as the control group.

Animal dose calculation

The rabbit dose was calculated based on the conversion factor of human dose to rabbit dose as shown below for lafutidine. The rabbit surface area considered for this research was 0.2 m^2, and the human surface area is 1.6 m^2. The human dose of lafutidine is 10 mg. The lafutidine animal dose is 1.25 mg.

Animal preparation

The animals assigned for the study were kept under observation in separate individual cages and deprived of any medication for the last fortnight. The animals were placed in an experimental room maintained at a temperature of 25° RH 45 and were exposed to a 12 hr light and dark cycle. The rooms were provided with 100%

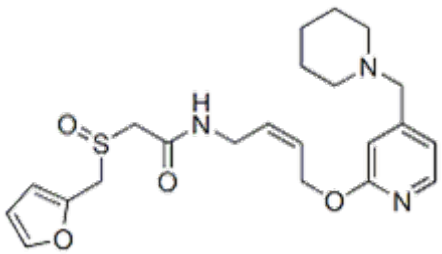

Figure 1: Molecular structure of lafutidine.

fresh air, uninterrupted power supply and water, and the animals were given a standard diet.

Experimentation

Before administration of the drug, the animals were fasted during night with free approach to water. The pre-calculated dose of the formulation was fed to the animals using a feeding tube and syringe.

Group A: Receive marketed formulation according to its body weight calculation. To ensure complete consumption of drug, the formulation was mixed with 1% CMC and fed 20 to 30 mL of water.

Group B: receives novel formulation equal to the dose to the animal body weight.

Group C: serve as a control.

Blood sampling procedure

After the administration of a dose, venepuncture, a 1 mL blood sample was from the marginal ear vein in a polypropylene container containing heparin at pre-set times of 0, 05, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, and 24 hr. The blood samples were thoroughly mixed with heparin to prevent blood clotting. The blood samples were centrifuged at 4000 RPM for 10 min for separation of plasma. The separated plasma was stored at -20°C until further analysis.

Analytical method adopted: Reverse phase high performance chromatography.

Table 1: Composition of optimized Lafutidine novel raft formulation.

Sl. No.	Name of the ingredient	Composition
1	Lafutidine	200 mg
2	Sodium alginate	0.52% w/v
3	Xanthan gum	1% w/v
4	HPMC K4M	1.67% w/v
5	$CaCl_2$	0.01% w/v
6	$NaHCO_3$	0.5% w/v
7	$CaCO_3$	2.5% w/v
8	Tri sodium citrate	0.3% w/v
9	Methyl and propyl paraben	0.2% w/v
10	Flavoring agent	Q.S.
11	Deionized water	Volume makeup to 100 mL

Dose: 10 mg/5 mL.

Specification of HPLC column

Mobile phase: 0.02 M phosphate buffer (2.72 g potassium dihydrogen phosphate and 3.4 g dipotassium hydrogen phosphate in 1000 mL) and acetonitrile at a ratio of 30:70 v/v

Preparation of lafutidine stock solution

50 mg of lafutidine was dissolved in 50 mL of methanol in a volumetric flask, which gave a 1000 µ/mL concentration. The solution was further diluted using the mobile phase to give a 10 µ/mL concentration.

Preparation of internal standard

The internal standard stock solution (diazepam) was prepared in a similar manner to give a 10 µ/mL concentration. Both solutions were run in HPLC to determine retention time.

HPLC procedure for method validation

Preparation of the calibration curve

A plasma standard solution of lafutidine was prepared by using the required amount of stock solution with 900 µL of plasma. Dilutions of 5, 10, 25, 50, 75, 100, 125, 150, and 200 ng/mL were prepared and subjected to HPLC. The plasma calibration curve was obtained by taking the concentration of the analyte versus the peak area ratio.

Sample preparation

For the separated plasma sample, an equal volume of 5% per chloric acid was used to precipitate any protein present in the sample which was then subjected to centrifugation for 2000 r.p.m. for 10 min. The samples were injected into the HPLC column for estimation.

Table 2: HPLC analytical method specification.

Instrument	HPLC Waters HPLC 2695
Column	C_{18} 250*4.5 mm 5 µSS
Flow rate	1 mL/min
Detector wavelength	UV 215 nm
Mobile phase	0.02 M phosphate buffer and acetonitrile at a ratio of 30:70 v/v.
Injection volume	50 µL
Run time	7 min
Mode	Isocratic

Table 3: Evaluation of optimized formulation.

Formulation	PH	*In vitro* gelation time (sec)	Floating lag time (sec)	Floating duration (hr)	Density (g/cm³)	Gel strength (g/cm²)	Viscosity (cps)	Drug content (%)
F3	7.34±0.73	6	17	>8	0.762±0.17	9.21	139	99.55±0.19

Quantitative estimation of drug

The concentration of drug in the sample can be determined by HPLC using a UV detector at its characteristic wavelength. The drug concentration can be calculated by interpolating the peak area of the blank plasma calibration curve over the spiked concentration range.

Specificity

To identify any interfering peaks due to the endogenous substance, present in plasma and was determined by spiking the randomly collected (n=6) blank plasma into the HPLC chromatogram.

Linearity

The best fit concentration that produces a detector response can be obtained from the regression equation and was found to have the following concentration range with correlation coefficient R^2. To estimate the concentration range for linearity, different concentrations of lafutidine (50% to 200%) were prepared in precipitated plasma along with an internal standard and a spiked HPLC column.

Accuracy

The accuracy of a method states the agreement of the mean test result over the nominal values used. Accuracy can be measured by taking three different concentrations (low, intermediate and high) and three determinations per concentration. It is expressed as a percentage.

Precision

The precision of a method can be stated as the degree to which individual test results agree with one another when applied to multiple samples. The precision of the method was measured by considering the percent coefficient variation of the low, intermediate and high concentration ranges of the sample for validation. This was measured by 3 determinations for at least 3 concentrations.

LOD

The lowest reliable concentration of drug to which the HPLC can detect but necessarily quantifiable. The recommended signal to noise ratio should be 1:3 and was considered the least nonzero value. It is expressed in ppm or ng/mL.

LOQ

The lowest concentration at which an instrument is able to detect and quantify a substance is referred to as its Limit of Quantification (LOQ). It is recommended that the ratio of noise to signal for LOQ be 1:10. It is expressed in ppm or ng/mL.

Evaluation of pharmacokinetic parameters in rabbit plasma

The evaluation of *in vivo* pharmacokinetic parameters helps in comparing the marketed formulation with that of the novel formulation.

C_{max} and T_{max}

Peak for plasma concentration and time to reach the peak concentration. These can be directly calculated from the graph of concentration vs. time plot.

Area Under Curve (AUC)

The important pharmacokinetic parameter, which helps to evaluate the formulation under consideration, is successful or not.

$t_{1/2}$ Known as biological half-life. It can be obtained from the elimination phase of plasma concentration vs. time. By plotting semi log C versus time.

Mean Residence Time (MRT)

The amount of time drug remained or spent in the body can be assessed by calculating the Mean Residence Time (MRT). MRT is a statistical approach that utilizes statistical moment theory, the mean residence time is considered as a statistical distributive curve, and can be calculated using the following equation

Table 4: ***In vitro*** **release of novel raft formulation.**

Sl. No.	time	%C DR
1	0.5	25.23±0.50
2	1	35.62±0.87
3	2	45.44±0.81
4	3	53.4±0.92
5	4	61.55±0.79
6	5	69.48±0.69
7	6	77.61±0.67
8	8	90.39±0.43
9	12	96.24±0.45

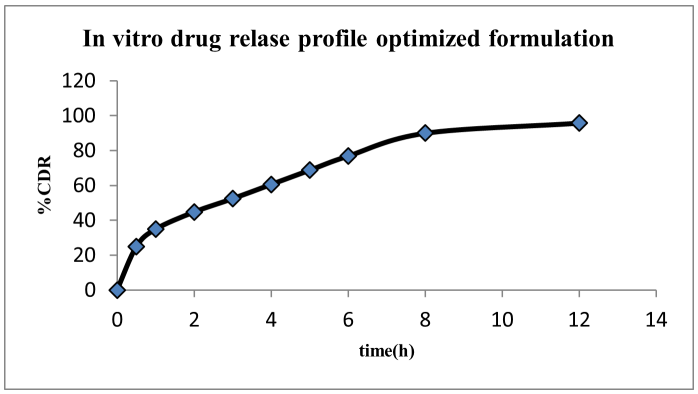

Figure 2: *In vitro* release of optimized novel raft formulation using 0.1 N HCl as dissolution media using type-II dissolution apparatus.

$$MRT = AUMC/AUC$$

Where,

AUMC is Area Under "First Moment" Curve. Can be calculated from the plot of plasma concentration of drug versus time (from zero to infinity).

$$AUMC = \int_0^{\infty} ct(t)dt$$

AUC is the area under the zero moment curves. It can be calculated from the concentration versus time using the trapezoidal method (from zero to infinity).

$$AUC = \int_0^{\infty} c(t)d$$

Statistical analysis

The results of obtained from pharmacokinetic parameter were statistically analysed using student *t* test ($p<0.05$) (paired two sample means) using data analysis add-in of Microsoft excel.

RESULTS

Formula optimization

17 experiments were carried out according to the Box Behnken Design, and the results were recorded. To optimise all of the responses at the same time, an optimization procedure using a desirability function was used. The following responses were used to create the desirability scale: buoyancy lag time (Y1), percent drug release at 1 hr (Y2), and cumulative percentage of drug release at 12 hr (Y3) (Y3). Y1 and Y2 had to be reduced, while Y3 had to be increased. The highest function value was obtained at A: 0.52 percent w/v, B: 1.67% w/v and C: 1.0% w/v, with a D value of 0.912. The resulting optimized formulation composition is given in Table 1.

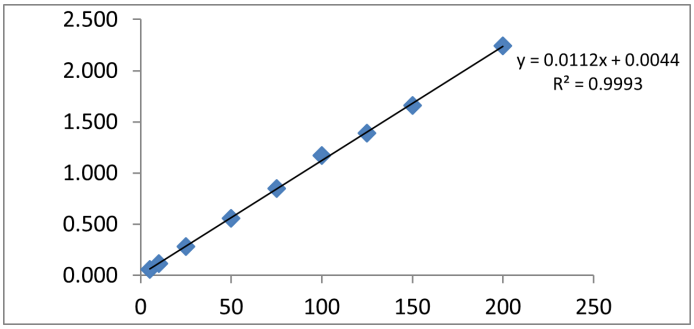

Figure 3: Standard calibration of lafutidine.

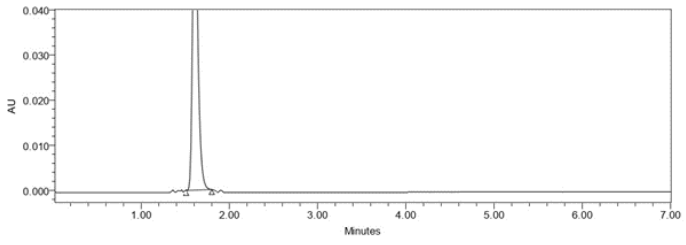

Figure 4: HPLC chromatogram of blank rabbit plasma.

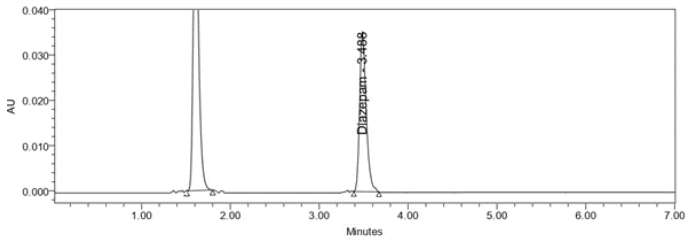

Figure 5: HPLC chromatogram of diazepam (internal standard) in rabbit plasma.

Evaluation of raft novel formulation

The evaluation of optimized formulation was carried out for enlisted tests and results are summarized in the Table 2. The *in vitro* dissolution was carried out in 0.1 N HCL as the formulation has to release the drug in gastric region and *in vitro* release study was given in Table 3, and Figure 2.

Table 2: *In vitro* release study of optimized formulation. The *in vitro* release was conducted as per procedure; the formulation was able to sustain release up to 12 hr and cumulative percent drug release was illustrated in Table 4 and the corresponding plot is given in Figure 1.

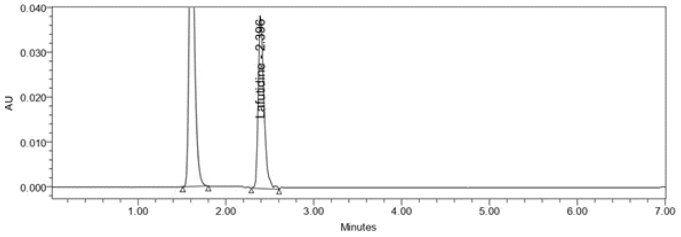

Figure 6: HPLC chromatogram of lafutidine in rabbit plasma

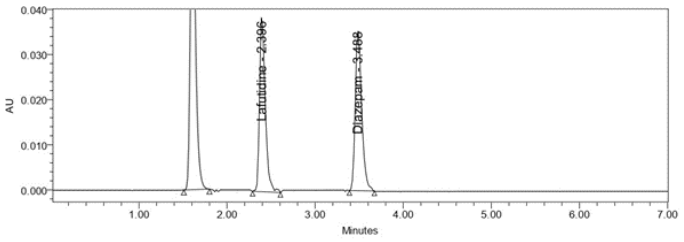

Figure 7: HPLC chromatogram of lafutidine and diazepam in rabbit plasma.

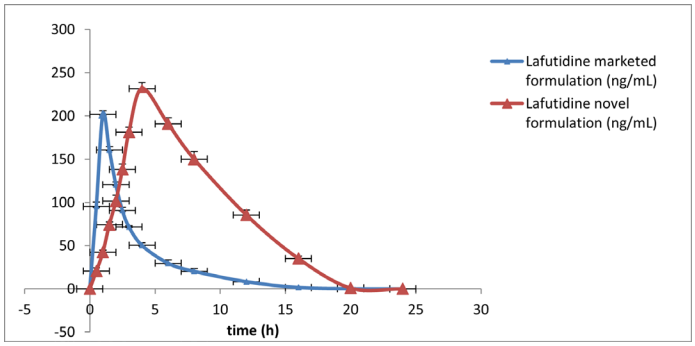

Figure 8: Plasma conc. vs.time of lafutidine novel raft gel and marketed product in rabbit plasma.

Table 5: Linearity results for lafutidine and IS.

Concentration	LAF Peak area	IS Peak area	Peak area ratios
5	8454	150160	0.0562±0.00
10	17152	150312	0.1133±0.00
25	42173	149936	0.2802±0.00
50	84002	152794	0.5546±0.01
75	128813	151744	0.8455±0.01
100	178689	150460	1.1688±0.04
125	210691	149630	1.3892±0.02
150	249674	149850	1.6577±0.01
200	336752	149318	2.2404±0.02

N=3 (Mean±SD).

Slope	0.1112
Intercept	0.0044
Corr. Coeff.	0.9993

Table 6: Plasma conc vs. time profiles of the lafutidine marketed product and novel raft formulation in rabbit plasma.

Time (h)	Lafutidine marketed formulation (ng/mL)	Lafutidine novel formulation (ng/mL)
0	0	0
0.5	95.42±0.57	20.56±0.64
1	201.73±0.19	42.24±0.39
1.5	160.76±0.32	74.35±0.48
2	120.5±0.27	101.71±0.59
2.5	90.72±0.40	138.05±0.24
3	71.84±0.82	181.2±0.79
4	50.26±0.34	231.66±0.46
6	29.6±0.72	190.81±0.28
8	20.27±0.64	150.07±0.53
12	8.24±0.92	85.52±0.10
[16]	1.5±0.85	35.05±0.24
20	0.1±0.12	1.01±0.71
24	0	0±0

Plasma concentration vs. time, each value represents the mean±SD, *n*=6.

Result obtained from kinetics of drug release study indicates the drug follows zero order kinetics (R^2 value 0.96609) with Higuchi mode (R^2 0.91413) with slope value less than 0.45 drug release mechanism. Thus polymer present in the formulation hydrates and swell due dissolution medium and drug diffuses through swollen polymer.

Analytical method results

Specificity

The HPLC chromatogram of blank plasma and lafutidine along with the internal standard is shown in Figure 3. The chromatogram revealed no interfering peaks, indicating the specificity of the method adopted.

Linearity

Standard calibration was generated for the linear concentration range given in Table 5. From the curve, it is clear that the peak area ratios obtained from lafutidine and the internal standard are proportional to the concentration. The concentration range used was 5-200 ng/mL. and curve linearly over this range, as shown

Table 7: Statistical treatment of Pharmacokinetics of the marketed and novel raft formulations of lafutidine in rabbit plasma (mean±SD, *n*=6).

Pharmacokinetic Parameter	Marketed formulation	Novel raft formulation	Calculated t value
C_{max} (ng/mL)	201.61±4.29	231.66±6.7	16.09***
t1/2 (h)	2.53±0.06	5.37±0.1	48.97***
Kel (h^{-1})	0.273±0.005	0.129±0.002	48.82***
AUC0-∞ (ng h/mL)	623.6±14.25	1999.31±41.1	65.25***
AUMC0-∞ (ng h/mL)	2292.11±110.57	14482.85±543.12	50.55***
MRT (h)	3.67±0.09	7.26±0.11	49.64***

*** student t test; SD: Standard deviation; c_{max}: peak plasma concentration; t_{max}: time to reach c_{max}; AUC_{0-24}: area under the curve from 0 to 24 hr; $AUC_{0-\infty}$: area under the curve from 0 hr to infinity; $MRT_{0-24:}$ mean residence time from 0 to 24 hr; $MRT_{0-\infty}$: mean residence time from 0 hr to infinity.

Figure 2. The regression coefficient equation y=0.0112x+0.0044 and R^2=0.9993.

Table 4: (Mean±SD, *n*=3) In *in vitro* release profile of novel raft formulation using 0.1 N HCL as dissolution media using type-II dissolution apparatus, value represents cumulative percent w/w drug release.

Accuracy

For accuracy measurement, the three concentration ranges used were 50, 100 and 150 ng/mL. The accuracy was expressed as percent recovery with percent relative standard deviation. (% RSD), percent accuracy was found to be 98.41% to 101.4%. The %RSD varies from 0.927 to 1.77.

Precision

For precision measurement, 100 (ng/mL) concentrations were selected. A inter and intraday precision of 96.61 to 101.40% and 89.38 to 91, 26 was found with % RSD varying from 1.83 to 0.82LOD and LOQ. The lowest concentration of analyte that is sufficient to provide a peak with a signal-to-noise ratio larger than 3:1 for LOD and 10:1. The lowest reliable limit was found to be 3 ng/mL as the concentration for LOD and 10 ng/mL for LOQ. The typical chromatogram of blank plasma spiked with lafutidine with internal standard indicating retention time is shown in Figures 4-7.

Pharmacokinetic study of lafutidine

All the pharmacokinetic study data were presented as mean and SD (*n*=6). The values are presented in Table 6. The lafutidine cumulative concentration was found to reach highest in during first hour in case of marketed formulation which indicates that it is well absorbed and elimination was exponential (Figure 8). The values of optimized novel formulation indicated drug was slow and reached maximum value during its fourth hour; this slow absorption was due to the formulation releasing the entrapped drug in a sustained manner.

DISCUSSION

The pharmacokinetic parameter lafutidine after oral administration was successfully evaluated using RP-HPLC, the value is expressed as mean and standard deviation displayed in Table 7. The C_{max} of the novel raft lafutidine formulation 231.66±0.46 ng/mL was found compared to the marketed lafutidine formulation 201.73±0.19 ng/mL. With corresponding T_{max} value of 4.0±0.06 h and 1±0.07 h. the mean $AUC_{0-\infty}$ for novel formulation was found to be 1989.79±1.02 ng.h/mL, and for the marketed lafutidine formulation, 623.18±0.55 ng.h/mL. This indicates that more amount drug is available in plasma. The mean residence time (MRT) was found to be 2.056 for the marketed formulation, whereas in case 7.0309, the MRT novel formulation was found to be more than 3 times more than marketed formulation. The difference between pharmacokinetics optimized formulation novel raft and markted formulation further evaluated using paired *t* test, the calculated *t* value (p<0.05) are significantly higher than table *t* values (Table 7). The table *t* value is 2.015 at 5 Degree of Fredom.

CONCLUSION

The developed HPLC method was found to be simple and reproducible for the estimation of drugs from biological samples and hence can be successfully applied for pharmacokinetic studies. From the *in vivo* PK study results, the pharmacokinetic parameters are significantly different for the marketed formulation compared to the novel formulation. The plasma concentration was attained rapidly in the case of the marketed formulation, whereas in the case of the novel formulation, there was slow release of the drug during its entire duration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High performance liquid chromatography; **CGRP:** Calcitonin gene related peptide; **AUC:** Area under curve; **BCS:** Biopharmaceutical Classification System; **MRT:** Mean residence time; **PK:** Pharmacokinetic; **IS:** Internal Standard; **%RSD:** Percent Relative Standard Deviation; **IAEC:** Institutional Animal Ethical Committee; **RH:** Relative Humidity, **CMC:** Carboxyl Methyl Cellulose; **BBD:** Box Behnken Design.

SUMMARY

This research work was conducted to analyse pharmacokinetic study of lafutidine raft formulation in comparison with conventional tablet using rabbit plasma. Lafutidine is an anti-secretory drug which prevent gastric secretion, due to its short half-life and absorption window it shows poor bioavailability. Lafutidine raft formulation was prepared using sodium alginate, xanthan gum, and HPMC K4M. The study was carried out using New Zealand rabbits. The plasma concentrations of drug were analysed using validated RP-HPLC method. The pharmacokinetic parameters such as C_{max}, T_{max}, Ke, AUC, and MRT were calculated. The data pharmacokinetic study was statistically analysed using paired student *t* test. From the statistical it was concluded; raft formulation has more bioavailability compared marketed formulation.

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