Section A-Research paper

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SIMULTANEOUS ESTIMATION OF H2 BLOCKERS GERD DOSAGE FORMS BY USING HPLC METHOD

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Abstract

The simultaneous analysis of Lafutidine and Domperidone Maleate in tablet form has been analysed using an HPLC method that has been developed and verified. At a flow rate of 1.0 mL/min and using UV detection at 222 nm, drugs were separated chromatographically using a Hypersil BDS C8 column (250 mm x 4.6 mm, 5) as the stationary phase and a mobile phase of phosphate buffer (pH adjusted to 4.5 with orthophosphoric acid):methanol:acetonitrile in the ratio 55:25:20 (v/v/v). Lafutidine had a retention time of 4.07 minutes, while domperidone took 6.13 minutes. The technique was found to be selective, with clearly distinguishable peaks for Lafutidine and Domperidone (resolution = 9.82). Linearity (R2 = 0.999) and accuracy (99.45-

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100.08% for Lafutidine and 99.20100.12% for Domperidone) as well as precision (%RSD 2%) characterise the suggested approach. The approach has been used to measure the effectiveness of commercial products, and those results have been found to be within acceptable ranges. Analysing tablet forms of Lafutidine and Domperidone is possible using this technique.

Keywords: Lafutidine, Analysis, Chromatograph, RP-HPLC, Domperidome

Introduction

From the literature survey, it was revealed that few spectrometric and chromatographic analytical methods have been developed for determination of eptifibatide in pharmaceutical preparations and biological fluids (7-11). Characterization of eptifibatide impurities during stability assays by using mass spectrometers coupled with a reverse phase gradient HPLC system has been performed by Wang *et al.* in 2003. Zhao *et al*. reported an isocratic RP-HPLC method for the assay of eptifibatide during drug stabilization studies (12). In this method, quantification of eptifibatide was achieved with UV detection at 220 nm (RT: 10 min). Kota *et al.* and Saksena *et al.* developed different reverse phase gradient HPLC methods for purity checking of synthetic eptifibatide substance after synthesis and purification procedures, but validation and applicability of these methods for marketed formulations were not reported (13, 14). Kota *et al.* used ACN and water as mobile phase. In this method, eptifibatide analysis time was 20 min. Saksena *et al.* presented method using ethanol and water as mobile phase (RT: 30 min). The drawbacks of the reported methods were the need for using gradient LC separation method with long run time and use of mass spectrometry that might not be universally available in laboratories due to its cost implications. Domperidone, 5- chloro- 1- [1- [3- (2- oxo- 2, 3- dihydro- 1Hbenzimidazol- 1- yl) propyl]- piperidin- 4- yl]- 1, 3- dihydro- 2H benzimidazol- 2- one (MW=425.9) acts by selectively antagonizing the peripheral dopaminergic D2 receptors in the gastrointestinal wall, thereby enhancing gastrointestinal peristalsis and motility and increasing lower esophageal sphincter tone. This increased gastrointestinal motility can facilitates the movement of acid contents further down in the intestine preventing reflux esophagitis and thereby controlling nausea and vomiting 1 . It is a official compound of B. P.2 . A survey of literature reveals that HPLC method is not available for estimation of the drug Domperidome in tablet dosage form.3 Domperidone is a dopamine-2 receptor antagonist. It acts as an antiemetic and a prokinetic agent through its effects on the chemoreceptor trigger zone and motor function of the stomach and small intestine. Unlike metoclopramide, it does not cause any adverse neurological symptoms as it has minimal penetration through the blood-brain barrier. It thus provides an excellent safety profile for long-term administration orally in the recommended doses. Domperidone is widely used in many countries and can now be officially prescribed to patients in the United States by an investigational new drug application for the treatment of gastroparesis and any condition causing chronic nausea and vomiting. In view of this additional clinical exposure of domperidone to a new generation of gastroenterologists and other specialists. Lafutidine, a histamine H(2)-receptor antagonist, inhibits gastric acid secretion during the daytime, however,

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the relationship between the plasma concentration and the drug response remains unclear. The aim of this study was to compare the pharmacokinetic and pharmacodynamic properties of lafutidine and famotidine following postprandial oral administration. After a lafutidine tablet (10 mg), famotidine tablet (20 mg), or water only (control) was administered, blood samples were taken and intragastric pH was measured. The plasma concentrations of lafutidine and famotidine were determined by HPLC, and the median intragastric pH values per 30 min were used as the degrees of gastric acid suppression. Data were analyzed based on a one-compartment pharmacokinetic model and a sigmoid E(max) pharmacodynamic model. Lafutidine plasma concentrations rapidly increased after administration; famotidine required some time to increase the plasma concentrations, requiring an absorption lag time in the pharmacokinetic model. Between the plasma concentration and DeltapH (the difference in intragastric pH by the drug vs. control), lafutidine showed an anticlockwise hysteresis loop which indicated equilibration delay between the plasma concentration and effect site, requiring an effect site compartment in the pharmacodynamic model; famotidine showed more parallel relationship. These results indicated that the pharmacokinetic and pharmacodynamic properties of lafutidine after postprandial oral administration were different from those of famotidine at least 4.5 h after dosing.

MATERIALS

The method was designed and validated using HPLC (Waters 2996 with PDA detector). The separation was performed using a column (Intersil C18 250 to 4,6 mm, 5µm), maintained at 40°C. The collecting of data was carried out using the software Empower.

Chromatographic Equipments and Conditions

The chemicals and reagents used were same as for previous method.

METHODOLOGY

Mobile Phase

The mobile phase as a blend of acetonitrile and buffer has been completed (buffer: 1ml Orthophosphoric Acid in one litre of water). Methanol: acetonitrile: water was produced and utilised as a diluent (50+30+20). During the chromatographic run, the gradient elution mode was followed at different times:

Preparation of Standard Solutions for Lafutidine and Domperidone[23]

The accurate weighing of about 1000 mg Lafutidine ..and 3000 mg of Domperidone Maleate has been put into separate 100 ml volumetric bottles containing 70 ml of diluent and their content has been dissolved by sonication. The solutions have been chilled to room temperature, diluted with the diluent to 100 ml and used as stock solution. Additional 1 mL of standard stock solutions for Laputidine and Domeperidone were transferred to separate 1000 ml flask, and diluent marking (10 µg/ml for Lafutidine and 30 µg/ml for Domperidone maleate) was made up of the volume.[20]

Preparation of Sample Solution (Lafutidine & Domperidone 10mg+30mg) [22]

The materials were weighed and combined with 20 capsules (label claim 10 mg + 30 mg). In a volumetric flask of 100 ml of 70 ml of diluent, the contents of five capsules were transmitted and

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sonicated 30 minutes with occasional vigorous shaking. The solution has been cooled to room temperature and the volume has been diluted to 100 ml. The solution has been filtered with a teflon filter syringe of 0.45µ. 1 ml of this solution was further diluted into 50 ml and blended (10 µg/ml + 30 µg/ml concentration) with diluents.

Optimization of Chromatographic Conditions and Method Development [24]

Several chromatographic cycles have been taken for individual medicines and their mix in various mobile phase combinations. A number of mobile phases have been explored, like acetonitrile and water, acetonitrile and buffer (KH2PO4, OPA buffer), methanol buffer. Finally, as a mobile phase for additional chromatographic studies with gradient elution, buffer (0.1 percent v/v OPA in water) and acetonitrile were utilised.

Method Validation [25]

The method validation was performed according to ICH parameter requirements, such as specificities, forced degradation, precision, precision, linearity, LOD, LOQ and analysis solution stability (ICH 1996, ICH 2003, ICH 2005).

i) System Suitability Study

In five replicates (solution preparation as indicated in the procedure 6.2.2.3 above), 10µl of standard preparation have been injected. For Lafutidine and Domeperidone, the chromatograms and the peak responses were measured. System appropriateness criteria like Rt, peak area, tail factor, theoretical plate and resolution.

ii) Specificity

The method specificity was tested by comparing blank solution chromatograms (10 µg/ml Lafutidine and 30 µg/mL Domperidone Maleate) with a mixed standard solution, and by injecting individual lafutidine (10 µg/ml) and domperidone maleate (30 µg/ml). The pitch purity was compared and the retention times of the principal peaks should not interfere.

iii) Assay of the Formulation

The injection of 10 µl sample solutions into duplicates measured the peak responses and the percentage test calculation for Lafutidine and Domeperidone was made using Equation 6.1.1.

iv) Precision

a) System Precision

In the HPLC system six replicate injections containing the 10 µg/ml mixture for Lafutidine and 30 µg/ml for Domperidone Maleate were administered. The average, SD and RSD percent were determined.

b) Method Precision

Six samples containing the known Lafutidine and Domeperidone amount were examined according to test procedure (10 µg/ml and 30 µg/ml). The percent test and relative deviation of the standard were determined.

c) Intraday and Interday Precision

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The intraday precision was evaluated by a two-hour, twelve-hour, daily examination at three distinct concentration levels of Lafutidine 7,5, 10, 12,5 µg/ml and Domperidone Maleate 30, 37,5 µg/ml. The interday precision investigation was also conducted for three separate days, i.e. day 1, day 2, and day three, at three levels of intraday concentration.

v)Accuracy (Recovery study)

The precise investigation of the method was carried out by the addition of the standard medicine to the preanalyzed sample at three levels 80%, 100% and 120%, and mean recoveries were determined. Powdered amount (614.31 mg) was carefully weighed and put to 100 mL of volumetric flask corresponding to 10 mg of Lafutidine and 30 mg of Domperidone Maleate. To this standard, Lafutidine and Domperidone Maleate were added to the labelled claim at 80%, 100% and 120% respectively. Each of them was then dissolved by sonication for 25 minutes in 50 ml of diluent with occasional vigorous shaking. The solutions were then refreshed to room temperature and up to 100 ml of diluent volume were produced. The solution was filtered by 0.45µ Teflon filter syringe and diluted and blended further. The recovery % was determined with the equation 6.1.2.

iii)Linearity and Range [26]

The linearity of the procedure was measured at nine levels of concentration. In standard stock solutions, standard solutions with varied concentrations of 0.1-15 µg/ml for Lafutidine and 0.3-45 µg/ml for Domperidone Maleate were produced. The HPLC apparatus injected 10 µl of each solution and the chromatogram peak area obtained was reported. Six duplicates were analysed at each level according to the approach proposed. The average area with its standard deviation and relative standard deviation of percentages of peak areas were determined at each level. The calibration curve was built by graphing medium area in curve versus drug concentration. Curve equation, and co-relation coefficients from calibration curves were calculated.

vi) Stability in Analytical Solution

The stability of Lafutidine and Domperidone Maleate in analytical solution was validated by storage in a refrigerator (8°C) and room temperature before and after 24 hours (10µg/ml of Lafutidine and 30 µg/mL of Domperidon Maleate, respectively). The percentage test has been determined from the Lafutidine and Domperidone Maleate peak regions.

vii) Limit of Detection (LOD) and Limit of Quantitation (LOQ) [27]

LOD and LOQ were determined from the slope (S) and the standard deviation (Ś) of reactions for lafutidine and domperidone maleate. The LOD and LOQ parameters were calculated with equations 6.1.3 and 6.1.4.

viii) Robustness

Samples of Lafutidine 10 µg/ml and Domperidone 30 µg/ml of each were made from sample stock solution and analysed in accordance with the technique proposed. Small yet planned alterations were done to assess its robustness, such as:

Column temperature - Changed to 35oC and 45oC (current column temperature maintained at 40oC)

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Flow rate – Flow rate change effect - Changed to 0.8 ml/min and 1.2 to ml/min (actual column flow rate was 1,0 ml/min)

Forced Degradation Study [24,28,29]

The investigation was designed to ensure that Lafutidine, Domperidone Maleate and their breakdown products were effectively separated. The stability indicating characteristics and specificity of the technique were assessed in forced degradation studies. To establish stability, sample powder and standard pharmaceuticals Lafutidine and Domaperidone Maleate and their mixture have undergone the same stress conditions to establish the suggested analytical test technique (ICH, 2003). The origin of the deterioration can be gained by comparing samples, individual medications and their combination chromatograms in stress circumstances, which supports the stability of the improved method for pharmaceuticals and pharmaceuticals. The peak purity study was measured using parameters for the purity angle and purity threshold. As Lafutidine and Domperidone Maleate are highly purified, acceptable standards for stressed samples were successfully met. The method therefore indicates stability. The capsule contents were correctly mixed together to transfer samples (625 mg) of Lafutidine equivalent to 10 mg (30 mg Domperidone Maleate) into a 100 ml volumetric flask holding 70 ml of diluent. The content has been sunken for up to 30 minutes, with an intermittent shaking, refrozen to room temperature and a diluent volume of up to 100 ml (sample stock solution). Similar solutions were developed for Lafutidine (10mg), Domperidone maleate (30 mg) and their mixture (standard stock solution). Forced degradation studies were conducted under the following conditions by treating the sample:

i) Acidic Degradation

In 50 ml volumetric flask with 30 ml of diluent, 5.0 ml of the above-mentioned stock solution was transferred. Added 5.0 ml 5 N hydrochloric acid to it and refluxated at 80°C for 30 minutes. The flasks were removed and refrigerated to room temperature after 30 minutes. With 5.0 ml of 5 N sodium hydroxide, the resultant solutions were neutralised. The volume was diluted to the mark and the components were adjusted. Filtered using 0.45µ membrane filter, the solution was analysed using the optimal technique.

ii) Alkaline Degradation

In the above sample stock solution, the 5 ml volumetric flask containing 30 ml of diluent was transferred into 50 ml. In addition, 5.0 ml of 5 N of sodium hydroxide was added and the solution refluxed at 80°C for about 30 minutes. The flasks were removed and refrigerated to room temperature after 30 minutes. With 5.0 ml of 5 N hydrochloric acid, the solutions were neutralised. The volume was diluted to the mark and the contents may be adjusted. Filtered using 0.45µ membrane filter, the solution was analysed using the optimal technique.

iii) Peroxide Degradation

A 5 mL volumetric bottle containing 30 ml of diluent of the aforesaid stock solution was transferred. In this 5.0 ml (30 percent) of hydrogen peroxide was added and the solution lasted

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30 minutes. The volume was diluted to the mark and the contents may be adjusted. Filtered using 0.45µ membrane filter, the solution was analysed using the optimal technique.

iv) Reduction

A 5 mL volumetric bottle containing 30 ml of diluent of the aforesaid stock solution was transferred. Added and soniced 5.0 ml of 5 ml 1N solution of sodium bisulphate for 30 min. The volume was diluted to the mark and the contents may be adjusted. The solution was filtered by a membrane of 0.45µ and analysed using the optimum procedure.

v) Thermal Degradation

The powder sample (621.4 mg) equivalent to 10 mg Lafutidine has been put into 100 ml of volumetric flask, retained and cooled in a hot air oven at 60 ° C for 24 hours. The volume was diluted to the mark and the contents may be adjusted. The solution was filtered by a membrane of 0.45µ and analysed using the optimum procedure.

vi) Photolytic Degradation

Sample powder (620.6 mg) corresponding to 10 mg of lafutidine was transferred to 100 ml of volumetric flask for 24 hours and exposed to photolytic conditions (1.2 million lux hours). The volume was diluted to the mark and the components were adjusted. The solution was filtered by a membrane of 0.45µ and analysed using the optimum procedure.

vii) Humidity

Sample powder (619.9 mg) equivalent to 10 mg Lafutidine was put in 100 ml volumetric flask and subjected for roughly 24 hours to 40°C/75 percent HR. The volume was diluted to the mark and the contents may be adjusted. The solution was filtered through 0.45µ membrane and the optimal procedure was assessed..

viii) Hydrolysis

The powder sample (618.9 mg) of Lafutidine equivalent to 10 mg was transferred in 100 ml of volumetric flask with 50 ml of water and allowed to stand up for 24 hours. The volume was diluted to the mark and the contents may be adjusted. The volume was diluted to the mark and the components were adjusted. The solution was filtered by a membrane of 0.45µ and analysed using the optimum procedure. Forced deterioration under acido, alkaline, peroxide and reduction was detailed for the sample of 5 ml of each standard stock solution. The solutions have been filtered though 0.45µ membrane, diluted and evaluated according to the optimal procedure. The standard solid for thermal, photolytic, humidity and hydrolysis were transmitted into separate volumetric flasks and subjected to forced degradation (10 mg Lafutidine, 30 mg Domperidone Maleate and 10 mg+30 mg) as specified for a sample. Diluted with diluent, filtered through 0.45µ membrane and assessed with the optimal procedure, the standard medicine after deterioration. Similarly samples and standards were diluted using a diluent (without degradation) and analysed by the optimised method.[30]

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RESULTS AND DISCUSSION

Preliminary Studies and Spectral Studies of Lafutidine and Domperidone [22]

Findings of pre-existing investigations were detailed in the preceding method.

The wavelength for this approach was picked from the individual and overlapping spectra of Lafutidine and Domperidone 217 nm.

Optimization of Chromatographic Conditions and Method Development [24]

In order to achieve the optimized chromatographic conditions to separate and quantify Lafutidine and Domperidone one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions. In fig 1 were taken to finalize chromatographic conditions. Few of them are mentioned in the table 1

TABLE: 1 VARIOUS TRIALS AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

TRAIL NO.	HPLC SYSTEM	CHROMATOGRAPHIC CONDITIONS	OBSERVATION	REMARKS
1	HPLC (WATER 2996 WITH PDA DETECTOR)	Mobile phase – Acetonitrile water Stationary phase- Zodiac c_{18} Injection – 10 µl Pump mode- Gradient Column Temperature- Ambient	No peaks eluted	Rejected
2	HPLC (WATER 2996 WITH PDA DETECTOR)	Mobile phase – Acetonitrile water Stationary phase- Zodiac c_{18} Injection – 10 µl Pump mode- Gradient Column Temperature- Ambient	Peaks were not sharp	Rejected
3	HPLC (WATER 2996 WITH PDA DETECTOR)	Mobile phase – Acetonitrile water Stationary phase- Zodiac c_{18} Injection – 10 µl Pump mode- Gradient Column Temperature- Ambient	Good peaks with proper resolution	Accepted

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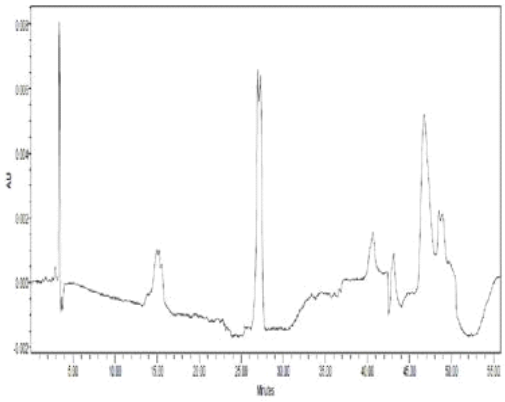
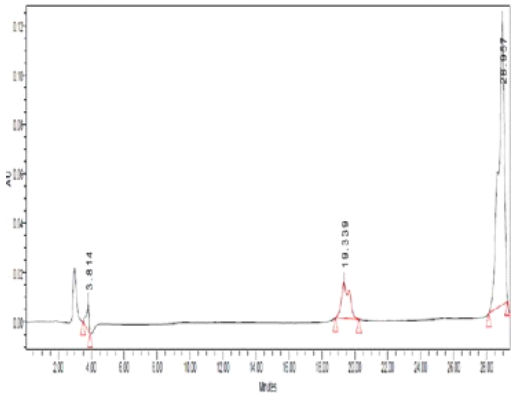
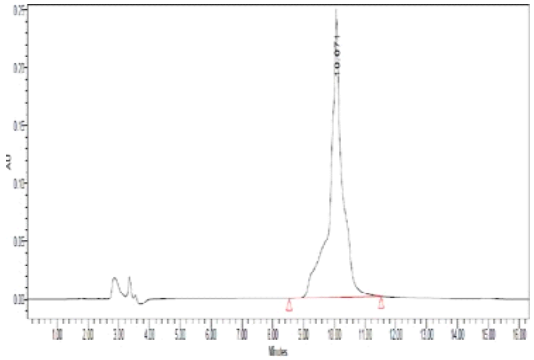


A: Trial 1: water Acetonitrile



B: Trial 2- water acetonitrile



C: Trial 3: OPA Buffer Acetonitrile

FIG 1: TRIAL METHOD FOR CHROMATOGRAMS FOR DEVELOPMENT

Method Validation [25]

i) System Suitability Study

The HPLC method was devised to determine the Lafutidine and Domperidone percentage test in their capsule forms. The blank, standard drug chromatograms alone and in their blend are presented in fig 2-4. The retention periods for Lafutidine and Domperidone were 19.9 min and 36.4 min and other characteristics such as resolution, tail factor and theoretical plates were found to be within acceptable limits (Table 2).

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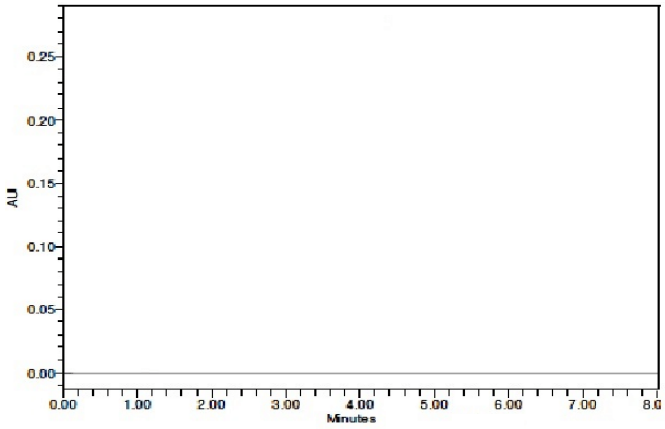

FIG 2 BLANK CHROMATOGRAM

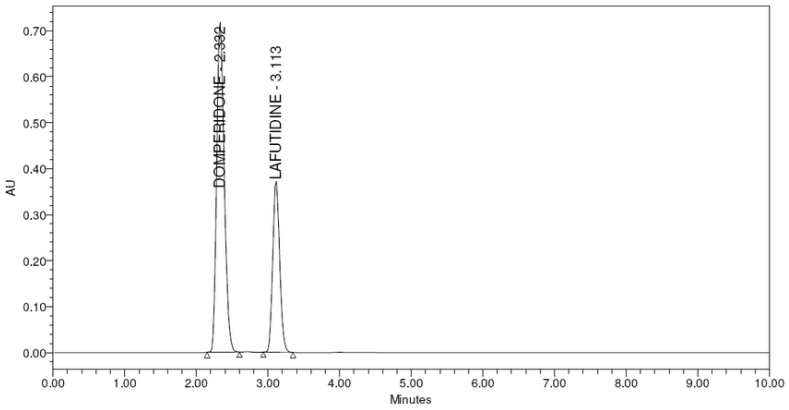

FIG 3: STANDARD LAFUTIDINE AND DOMPERIDOME OF CHROMATOGRAM

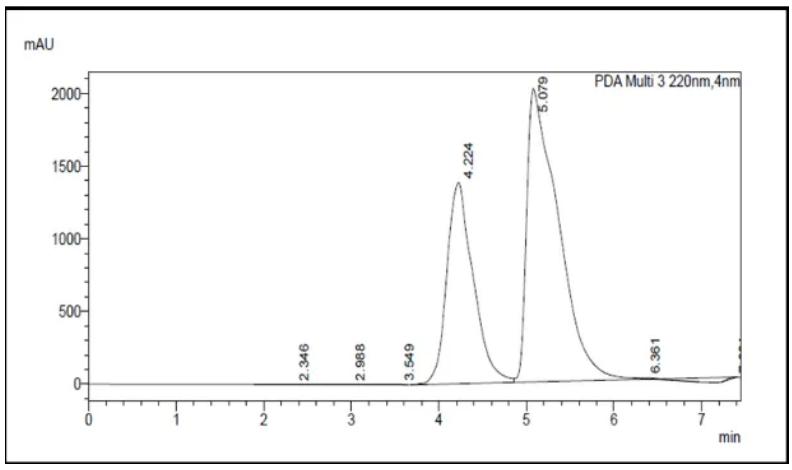

FIG 4: LAF & DOM CHROMATOGRAM

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TABLE 2: SYSTEM SUITABILITY PARAMETERS

S.NO	NAME OF DRUG	RETENTION TIME	AREA	USP RESOLUTION	USP TAILING	USP PLATE COUNT
1	**LAFUTIDINE**	19.993 ± (0237)	350760 ±(1015.45)	----------	0.9697 ± (0.07894)	8264.4 ±(134.43)
2	**DOMEPERIDONE**	36.41 ± (0.378)	914459 ± (1389.4)	28.36 ± (0.44)	1.1464 ± (0.0045)	52647.7 ±(1457.07)

ii) Specificity [26]

The lack of extra peaks in the chromatogram suggests that excipients are not interfering. There was no blank interference in analytical peak retention. The maximum purity of the sample solution was compared with the standard solution which shows the homogeneous peaks. The pure plots in figures 5 & 6 show the homogeneity of the peaks.

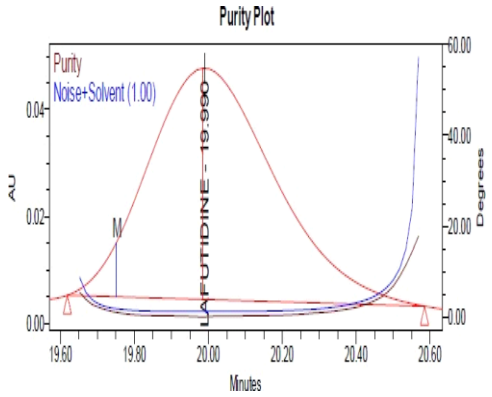

FIG 5 LAF PEAK PURITY PLOT

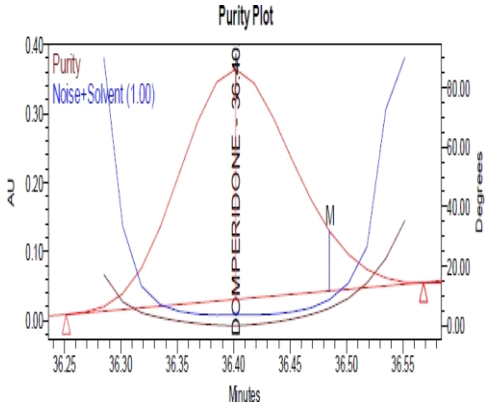

FIG 6: PEAK PURITY OF DOM

iii) Assay of Marketed Formulations

Two separate marketing capsules (Lafukem-D and Lafutax-D) formulations were analysed and a percentage test was found. Table 3 shows the results of the test and figures 7 and 8 show the chromatograms for the sample. The average percentage of Lafutidine and Domperidone in Lafutax-D were 99.32% and 100.27% respectively. The average percentage of Lafutidine and Domperidone tests for Lafukem-D were 100.7% and 99.92% respectively.

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TABLE 3 MARKETED FORMULATION ASSAY

MARKETED FORMULATION	RETENTION TIME OF LAFUTIDINE	AREA	% ASSAY LAFUTIDINE	RETENTION TIME OF DOMPERIDONE	AREA	% ASSAY DOMPERIDONE
LAFUTAX D	19.50	329765	99.32%	36.28	919132	100.27%
MEAN		3167458			916845	
		322878			9147988.4	99.92%
LAFUKEM D	19.50	328145		36.28	910523	
MEAN		326427	100.7%		919507	
		327282.6			919404	

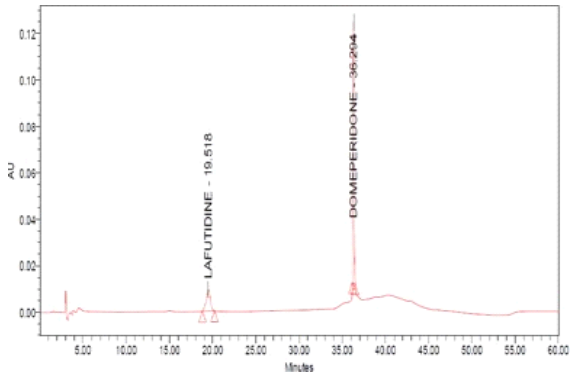

FIG 9 CHROMATOGRA, OF LAFUTAX –D

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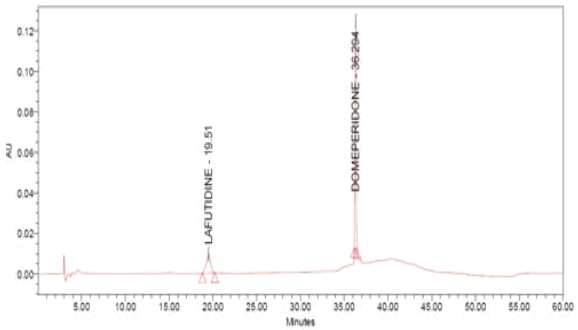

FIG 10 CHROMATGRAM OF LUFUKEM –D n

iv) Precision

a) System Precision

System precision was achieved by measuring the peak response in six replicates for standard medication solutions. Table 4 shows peak reactions, average, default and percent relative standard deviation (RSD) for Lafutidine & Domeperidone and has been found well within acceptable standards.

b) Method Precision

The procedure accuracy was carried out in six replicates by measuring the peak response for sample solutions. The percentage test for Lafutidine & Domeperidone was calculated in 6 samples and the percentage RSD was computed and reported in table 5 Figure 11 shows representative chromatograms for technique accuracy.

TABLE 5: PRECISION DATA SYSTEM

SR.NO	PEAK AREA OF LAFUTIDINE	PEAK AREAS OF DOMPERIDONE
1	307685	915002
2	310743	910474
3	310647	904190
4	310214	915884
5	315670	912759
6	3181770	919016
MEAN	311862	912876
SD (±)	3270.127	5174.68
% RSD	1.048	0.645

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TABLE 6: PRECISION DATA METHOD

SR.N0	% ASSAY OF LAFUTIDINE	% ASSAY OF DOMPERIDONE
1	99.4	99.2
2	99.3	98.4
3	99.2	99.6
4	100.4	100.4
5	98.5	99.1
6	100.52	100.6
MEAN	99.62	99.654
SD (±)	0.649814	0.6801
% RSD.	0.646798	0.677245

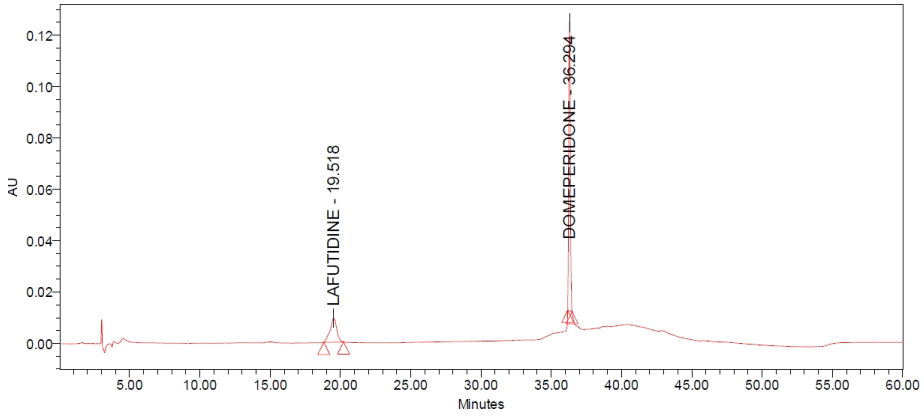

FIG 11 REPRESENTATIVE OF METHOD PRECISION

c) Intraday and Interday Precision

For Lafutidine, (7.5, 10, 12.5 µg/ml) intra-day precisions was found to be 0.1430, 0.4870, 0.47% and for Domperidone (22.5, 30, 37.5 µg/ml) to be 0.06, 0.43, 0.02%. In interday precision the percentage RSD was determined to have been of 0.1449, 0.4869, 0.49 percent for Lafutidine (7.5,10, 12.5 µg/ml) and 0.25, 0.08, 0.09 percent for Domperidone (22.5, 30, 37.5 µg/ml), respectively. RSD percentage was found well within acceptable ranges in intraday and interday trials. The results were reported in Tables 7 & 8.

v**) Accuracy (Recovery Studies)**

The accuracy investigation has been carried out by a retrieval of the additional Lafutidine and Domperidone standards at three distinct 80 percent levels, 120 percent level of label claim and the percentage of retrieval, standard deviation and RSD percentage, calculated and shown in Table 7 The average recovery percentage was 99.27% and 99.43% at the level of

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80%, 99.30% and 99.47% at 80% and 99.27% and 98.69% at the level of 120% for Lafutidine and Domperidone respectively. The findings of the recovery demonstrate that the procedure is precise to determine.

vi**) Linearity and Range**

Lafutidine and Domperidone linearity were determined to be 0,1 to 15 µg/ml, 0.3 to 45 µg/ml, with coefficient values of correspondence (r2) of 0.99984 to Lafutidine and 0.99866 to Domperidone respectively. A diagram was drawn using X axis concentration and a mean area was computed for Y axis peak and coefficient of correlation.The linearity chromatograms are given.

TABLE 7 INTRADAY PRECISION

	LAFUTIDINE						**DOMPERIDONE**			
S.NO	**CON (µg/ml)**	**AREA**	**MEAN**	**SDA**	**% RSD**	**CON (µg/ml)**	**AREA**	**MEAN**	**SDA**	**% RSD**
1	7.5	2366879	236872.4	341.12	0.1430	22.5	694889	695098	423.57	0.6
		236130					695043			
		237583					695881			
2	10	405969	4017899	1948.14	0.4870	30	916297	912207.8	3814.12	0.47
		396721					908642			
		391255					911470			
3	12.5	404219	401765.9	1906.05	0.47	37.5	1154078	1154809	233.71	0.03
		405781					1154624			
		399387					1154978			

TABLE 8 INTERDAY PRECISION

		LAFUTIDINE						DOMPERIDONE			
S.NO	DAY	CON (µg/ml)	AREA	MEAN	SDA	% RSD	CON (µg/ml)	AREA	MEAN	SD	% RSD

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1	DAY 1	7.5	236798	236874.4	616.12	0.1449	22.6	694971	695097	1687.15	0.25
	DAY 2		237814					693871 6			
	DAY 3		2368704					694240			
2	DAY 1	10	315082	401748	1958.36	0.4869	30	912546	914368	814.20	0.08
	DAY 2		316834					914076			
	DAY 3		317206					913785			
3	DAY 1	12.5	402894	401768.7	1907.77	0.49	37.5	1153897	1154359	1006.8	0.09
	DAY 2		399586					115478 4			
	DAY 3		399358					115269			

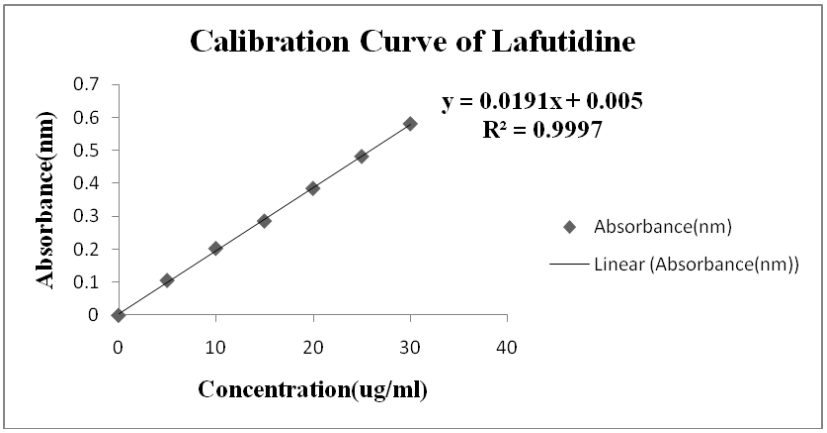

FIG 12 LAFUTIDINE CALIBRATION CURVE

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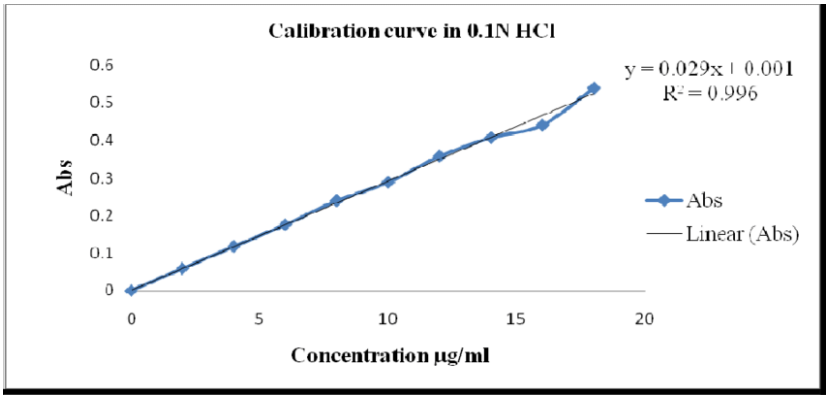

FIG 13 DOMPERIDONE OF CALIBRATIONS

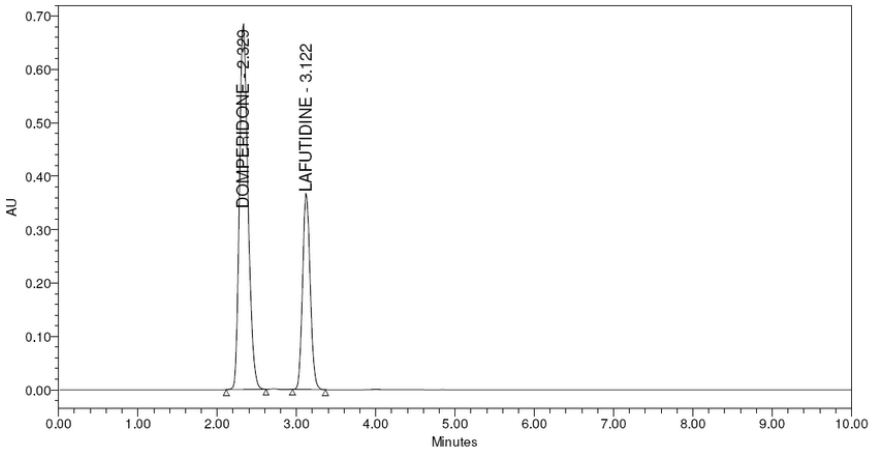

FIG 14 REPRESETATIVE OF LINEARITY

TABLE 9 RECOVERY STUDY

RECOVERY LEVEL %	80%		100%		120%	
	LAF	DOM	LAF	DOM	LAF	DOM
AMOUNT PRESENT (MG)	10	30	10	30	10	30
	10	30	10	30	10	30
	10	30	10	30	10	30
AMOUNT PF STD. ADDED (MG)	8.1	24.25	10.2	30.4	12.5	36.2
	8.24	24.0	10.2	30.3	12.2	36.1
	8.23	24.1	10.2	30.2	12.1	36.14
	8.01	24.10	10.06	30.25	11.95	35.57

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AMOUNT OF STD. RECOVERED IN MG	**8.23**	**24.10**	**10.8**	**30.14**	**12.14**	**35.75**
	8.17	**23.78**	**10.04**	**29.75**	**11.98**	**35.75**
% RECOVERY	**98.89**	**99.30**	**99.78**	**100.32**	**99.87**	**99.10**
	99.45	**100.45**	**99.56**	**99.45**	**99.45**	**98.25**
	99.35	**98.65**	**98.40**	**98.47**	**98.47**	**98.78**
MEAN RECOVERY	**99.24**	**99.43**	**99.25**	**99.75**	**99.25**	**98.65**
SD	**0.34**	**0.90**	**0.78**	**0.87**	**0.62**	**0.40**
%RSD	**0.32**	**0.90**	**0.78**	**0.87**	**0.65**	**0.41**

TABLE 10 RANGE AND LINERAITY

SR.NO	**LAFUTIDINE** (µg/ml)	**MEANS PEAKAREA**	DOMPERIDONE (µg/ml)	**MEAN PEAK AREA**
1	**0.00**	**0.00**	**0.00**	**0.00**
2	**0.10**	**2468**	**0.30**	**64879**
3	**0.50**	**15298**	**1.51**	**50397**
4	**1.01**	**27833**	**3.04**	**95647**
5	**2.52**	**75418**	**7.54**	**234736**
6	**5.04**	**15592**	**15.08**	**450934**
7	**7.56**	**238597**	**22.60**	**696475**
8	**10.09**	**325034**	**30.14**	**915438**
9	**12.65**	**402045**	**37.40**	**1153305**
10	**15.12**	**488045**	**45.25**	**1397987**

vii) Stability in Analytical Solution

In analytical solution, the stability of Laputidine and Domperidone was established by testing the sample in a refrigerator (8 degrees C) and room temperature before and after 24 hours. There was no significant variation in the percentage test of both medicines before and after 24 hours at refrigerator and room temperature. This indicates that the analytical

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solutions are stable. As a percentage test for Lafutidine and Domperidone, the solutions were stable for up to 24 hours at a cooler and 25°C ambient temperature.

Conclusion

The USFDA and ICH recommendations have advised the requirement of stability test data to evaluate the impact of various environmental conditions on the quality of a drug substance and a drug product over time. The choice of the appropriate formulation and container as well as the correct storage and shelf life, vital in regulatory paperwork, depend on molecular stability. An analysis method is described as a method for quantifying the active components precisely without interfering with degradation products, process contaminants, excipients or other potential impurities. It must also be confirmed to show that impurities that are specific to new medication ingredients do not interfere or are isolated from degradation products defined or undetermined in the drug product.

REFERENCES

1. Katz, P. O., Dunbar, K. B., Schnoll-Sussman, F. H., Greer, K. B., Yadlapati, R., & Spechler, S. J. (2022). ACG clinical guideline for the diagnosis and management of gastroesophageal reflux disease. *The American journal of gastroenterology*, *117*(1), 27-56.
2. Fejzo, M. S., Trovik, J., Grooten, I. J., Sridharan, K., Roseboom, T. J., Vikanes, Å., ... & Mullin, P. M. (2019). Nausea and vomiting of pregnancy and hyperemesis gravidarum. *Nature reviews Disease primers*, *5*(1), 62.
3. Amle, V. S., Rathod, D. A., Keshamma, E., Kumar, V., Kumar, R., & Saha, P. (2022). Bioactive Herbal Medicine Use for Eye Sight: A Meta Analysis. *Journal for Research in Applied Sciences and Biotechnology*, *1*(3), 42-50.
4. Katz, P. O., Gerson, L. B., & Vela, M. F. (2013). Guidelines for the diagnosis and management of gastroesophageal reflux disease. *Official journal of the American College of Gastroenterology| ACG*, *108*(3), 308-328.
5. Bharucha, A. E., Kudva, Y. C., & Prichard, D. O. (2019). Diabetic gastroparesis. *Endocrine reviews*, *40*(5), 1318-1352.
6. Kumar, R., Singh, A., & Painuly, N. (2022). Investigation of in-vitro anti-oxidant & anti-ulcer activity of polyherbal medicinal plants. *Journal of Pharmaceutical Negative Results*, 2077-2088.
7. Grover, M., Farrugia, G., & Stanghellini, V. (2019). Gastroparesis: a turning point in understanding and treatment. *Gut*, *68*(12), 2238-2250.
8. Mishra, A., Singh, Y., Singh, R., Kumar, R., Shukla, S., Kumar, R., ... & Pol, S. L. (2022). Ethano-pharmacology activity & Antioxidant activity of Centella asiatica Plant Parts. *NEUROQUANTOLOGY*, *20*(11), 7562-2.

9. Johnson, M. A., Moore, K. H., Yuen, G. J., Bye, A., & Pakes, G. E. (1999). Clinical pharmacokinetics of lamivudine. *Clinical pharmacokinetics*, *36*, 41-66.
10. Peters, M. G., Hann, H. W., Martin, P., Heathcote, E. J., Buggisch, P., Rubin, R., ... & Brosgart, C. L. (2004). Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology*, *126*(1), 91-101
11. SHAFQAT ZAIDI, R. K., MEHRA, D., SACHIN, T., & ROSHAN, K. A. D. (2021). Effect of Kalahari Cactus Extract on Appetitte, Body Weight And Lipid Profile In Cafeteria Diet Induced Obesity In Experimental Animal. *Annals of the Romanian Society for Cell Biology*, *25*(6), 13976-13987.
12. Fule, R., & Amin, P. (2014). Development and evaluation of lafutidine solid dispersion via hot melt extrusion: Investigating drug-polymer miscibility with advanced characterisation. *asian journal of pharmaceutical sciences*, *9*(2), 92-106.
13. Toida, M., Kato, K., Makita, H., Long, N. K., Takeda, T., Hatakeyama, D., ... & Shibata, T. (2009). Palliative effect of lafutidine on oral burning sensation. *Journal of oral pathology & medicine*, *38*(3), 262-268.
14. Kumar, R., Saha, P., Kahwa, I., Boateng, E. A., Boateng, P. O., & Nyarko, R. O. (2022). Biological Mode of Action of Phospholipase A and the Signalling and Pro and Anti Inflammatory Cytokines: A Review. *Journal of Advances in Medicine and Medical Research*, *34*(9), 1-10
15. Tanaka, M., Banba, M., Joko, A., & Moriyama, Y. (2001). Pharmacological and therapeutic properties of lafutidine (stogar and protecadin), a novel histamine H2 receptor antagonist with gastroprotective activity. *Nihon Yakurigaku zasshi. Folia Pharmacologica Japonica*, *117*(6), 377-386.
16. Patel, R. K., Gangwar, D., Gupta, H., Sharma, N., & Kumar, R. (2023). Plants Alkaloids Based Compound as Therapeutic Potential for Neurodegenerative. *Journal for Research in Applied Sciences and Biotechnology*, *2*(2), 14-26.
17. Subramanian, M., Keshamma, E., Janjua, D., Kumar, D., Kumar, R., Saha, P., ... & Rao, S. (2022). Quality Risk Management Approach for Drug Development and Its Future Prospectives. *Journal for Research in Applied Sciences and Biotechnology*, *1*(3), 166-177.
18. Kato, S., Tanaka, A., Kunikata, T., Umeda, M., & Takeuchi, K. (2000). Protective effect of lafutidine against indomethacin-induced intestinal ulceration in rats: relation to capsaicin-sensitive sensory neurons. *Digestion*, *61*(1), 39-46
19. Awuchi, C. G., Saha, P., Amle, V. S., Nyarko, R. O., Kumar, R., Boateng, E. A., ... & Asum, C. (2023). A Study of Various Medicinal Plants used in Ulcer Treatment: A Review. *Journal for Research in Applied Sciences and Biotechnology*, *2*(1), 234-246.
20. Awuchi, C. G., Amagwula, I. O., Priya, P., Kumar, R., Yezdani, U., & Khan, M. G. (2020). Aflatoxins in foods and feeds: A review on health implications, detection, and control. *Bull. Environ. Pharmacol. Life Sci*, *9*, 149-155.

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21. Rosca, A. E., Iesanu, M. I., Zahiu, C. D. M., Voiculescu, S. E., Paslaru, A. C., & Zagrean, A. M. (2020). Capsaicin and gut microbiota in health and disease. *Molecules*, *25*(23), 5681.
22. Higuchi, K., Umegaki, E., Watanabe, T., Yoda, Y., Morita, E., Murano, M., ... & Arakawa, T. (2009). Present status and strategy of NSAIDs-induced small bowel injury. *Journal of gastroenterology*, *44*, 879-888.
23. Das, S., Singh, V. D., Bind, A., Gupta, S. P., & Kumar, R. Evaluation of antiulcer, antioxidant potency of manuka honey and cinnamon powder mixture in Wistar rats using experimental rat models.
24. Keshri, S., Kumar, R., Kumar, D., Singhal, T., Giri, S., Sharma, I., & Vatsha, P. (2022). Insights Of Artificial Intelligence In Brain Disorder With Evidence Of Opportunity And Future Challenges. *Journal of Pharmaceutical Negative Results*, 10853-10867.
25. Afzaal, M., Saeed, F., Ateeq, H., Akhtar, M. N., Imran, A., Ahmed, A., ... & Awuchi, C. G. (2023). Probiotics encapsulated gastroprotective cross- linked microgels: Enhanced viability under stressed conditions with dried apple carrier. *Food Science & Nutrition*, *11*(2), 817-827.
26. Kumar, R., & Saha, P. (2022). A review on artificial intelligence and machine learning to improve cancer management and drug discovery. *International Journal for Research in Applied Sciences and Biotechnology*, *9*(3), 149-156.
27. Chaudhary, H., Sagar, S., Kumar, R., Bisht, V., & Butola, K. (2022). Herbal Essential Oil use as Ulcer Protective Activity: A Systematic Review. *Journal for Research in Applied Sciences and Biotechnology*, *1*(5), 86-101.
28. Grover, M., Farrugia, G., & Stanghellini, V. (2019). Gastroparesis: a turning point in understanding and treatment. *Gut*, *68*(12), 2238-2250.