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DEVELOPMENT AND VALIDATION OF RP-UHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAFUTIDINE AND DOMPERIDONE IN PHARMACEUTICAL TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, selective, sensitive, linear, precise and accurate RP-UHPLC method was developed and validated for simultaneous estimation of Lafutidine and Domperidone in pharmaceutical tablet dosage form. Separation of the drugs was achieved on a reverse phase by shim-pack C18: 250 x 4.6 mm, 3µm, column at 30˚c temperature using a mobile phase consisting of [Methanol: Ammonium Acetate Buffer pH 4.8, 20mM (75:25% v/v)] at a flow rate of 0.7 ml/min was employed. The RP- UHPLC detection wavelength was 220 nm and 10µL of sample was injected. The linearity was found for Lafutidine (35- 60 µ g mL-1) and Domperidone (105-180µ g mL-1) with a correlation coefficient of 0.999. Retention times were 4.2 min and 5.0 min for Lafutidine and Domperidone respectively. The method was validated as per the ICH guidelines for its selectivity, system suitability study, specificity, linearity, range, precision, accuracy, limit of detection, limit of quantification, robustness, ruggedness, assay. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was successfully employed for routine quality control analysis of Lafutidine and Domperidone in Pharmaceutical formulation.

KEYWORDS: Ultra-High-Performance Liquid Chromatography, Linearity, Precision, Accuracy, Calibration.

INTRODUCTION

Lafutidine (LAF) as shown in Fig. 1, a second generation H2 receptor antagonist having multimodal mechanism of action. It has been reported that the gastro protective effect of LAF is independent of its acid anti-secretory activity.[1] LAF not only suppresses gastric acid secretion, but also has cytoprotective properties by the virtue of its property to induce the collagen synthesis in the gastric mucosa.[2] In addition to being a potent H2 receptors antagonist LAF also activates capsaicin-sensitive afferent neurons and stimulates the release of calcitonin gene-related peptide (CGRP), which inhibits acid secretion and stimulates mucosal blood flow.[3-5] It is also found to stimulate mucin biosynthesis and promote the reconstitution of damaged mucosa (2). Chemically it is 2-(furan-2-ylmethylsulfinyl)-N- [4-[4(piperidin-1-ylmethyl) pyridin-2-yl] oxybut-2-enyl] acetamide.[6]

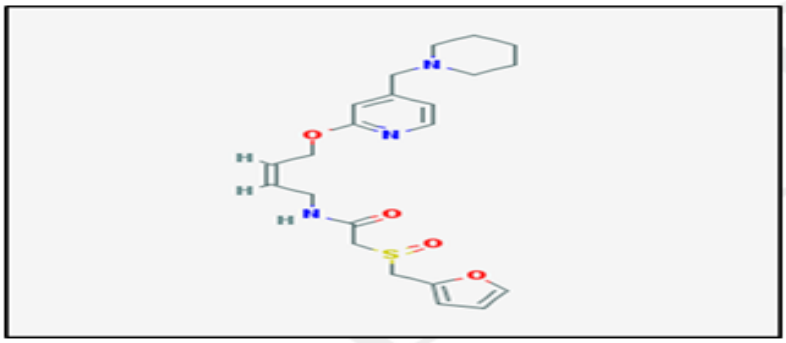

Fig. 1: Chemical Structure of Lafutidine (LAF).

Domperidone (DOM) as shown in Fig. 2 is a dopamine antagonist with antiemetic properties. It stimulates gastro-intestinal motility and is used as an antiemetic for the short-term treatment of nausea and vomiting. DOM is known chemically as 5-chloro-1-[1-[3-(2, 3dihydro-2-oxo-1H-benzimidazol-1-yl) propyl]-4-piperidinyl]-1, 3-dihydro-2H-benzimidazol2-one.[7-9]

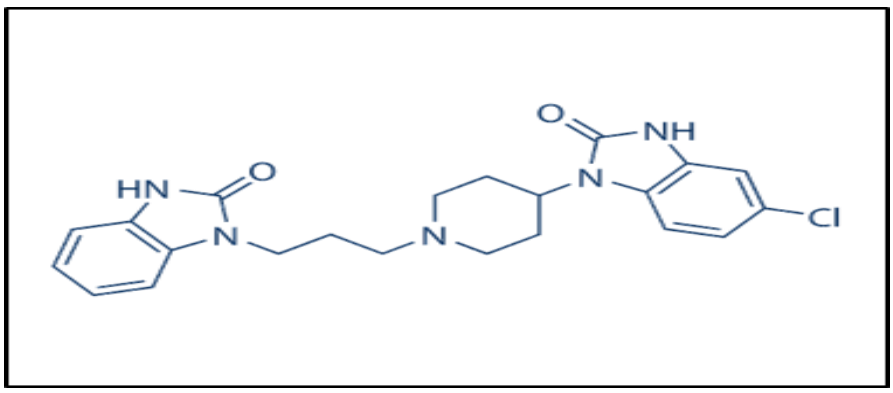

Fig. 2: Chemical Structure of Domperidone (DOM).

Analytical methods are needed to characterizethe drug component and composition during all phase of analysis. Early phase method must support change in synthetic rout, doses form, and elucidate the structure and level of impurity.[10-11] In later phase more focuses on development of robust method for release and stability evaluation. In the analysis phase many of the simple and instrument based analytical method were used however the most widely used method for quality assurance is based on the spectrophotometry and chromatography. In most quantitative analysis where a specification component is targeted in presence of sample matrix therefore, isolation and separation of the component is required preceding quantitative analysis which is usually achieved using chromatographic quantitative analysis.[12] In case where matrix interference is not observed quantitative measurement made based on the spectroscopic and titration method.

A literature survey revealed that methods were available for the determination of the LAF by LC- MS in human plasma,[13-14] HPTLC method,[15] UV Spectrometry method[16] and for the determination of DOM by HPLC.[17-18] Few methods for the determination of LAF and DOM in combination by HPLC[19-23], and Spectrometry[24,25] were available. There is no method reported in Lafutidine and Domperidone combination via ultra-high performance liquid chromatography. So that need was felt, to develop these new methods to analyze the drugs simultaneously.This manuscript describes the development and validation of RP-UHPLC method for simultaneous estimation of Lafutidine and Domperidone in pharmaceutical tablet dosage form. Advantage of RP-UHPLC method for traditional method: highest resolution, greatest achievable sensitivity, fastest analysis speeds, less solvent use, higher throughput and faster run times and column packed with sub 2-µm particle size. A successful attempt has been made to estimate two drugs simultaneously by RP-UHPLC method.

The present work demonstrates to develop and validation of economically viable, highly efficient, simple, accurate, reproducible and fast method for the simultaneous estimation of Lafutidine and Domperidone in pharmaceutical tabletdosage form by RP-UHPLC method.

MATERIAL AND METHOD

Drug: Lafutidine and Domperidone were generously gifted by "Swapnroop Drug and Pharmaceuticals Aurangabad" and the formulation containing Lafutidine 10 mg and Domperidone 30 mg tablet were purchased from a local pharmacy with the brand name LAFAXID-D.

Reagents & Chemicals: All the chemicals used were of analytical grade and HPLC grade procured from M/s Merck, Germany. The chemicals used for the study were.

- Acetic Acid (HPLC grade).
- Water (HPLC grade).
- Methanol (Analytical grade).
- Ammonium Acetate (Analytical grade).

Instrumentation and chromatographic conditions: Shimadzu Ultra High Performance Liquid Chromatography Nexera X2 (Japan) consisted of a binary pump, an automatic injector, variable wavelength detector, and a column oven was used for analysis. Data were processed by usingLab solution 6.82-ST1 software. Chromatographic separation of Lafutidine and Domperidone were performed using Shim-pack C18 (4.6 mm × 250 mm, 3µm) column.column oven temperature of 30°C andeluted with mobile phase flow rate of 0.7 ml/min. The mobile phase was absolute Methanol and Ammonium acetate buffer PH (4.8), 20mM which was filtered 0.2µm syringe filter anddegassed in ultrasonic bath before use. Measurements were done with injection volume of 10µl and detector wavelength at 220 nm.

Preparation of standard Lafutidine solution: 50 mg of standard Lafutidine was weighed accurately and transferred into a 100 mL volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (500 µ g mL-1). Further dilution was made by pipetting 10mL of standard stock into a 100mL to acquire 50 µ g mL-1 solutions.

Preparation of standard Domperidone solution: 190.89 mg of standard Domperidone equivalent to 150 mg of Domperidone Maleate was weighed accurately and transferred into a 100mL volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (1500 µ g mL-1). Further dilution was made by pipetting 10mL of standard stock into a 100mL to acquire 150 µ g mL-1 solutions.

RESULTS AND DISCUSSION

Method validation: The method was validated according to the ICH guideline. The following validation characteristics were addressed: system suitability study, specificity, linearity, precision (intraday/ inter-day precision), accuracy, robustness, ruggedness, detection limit, quantitation limit, and assay.

System suitability study: System suitability solution was prepared from daily using stocksolution, for that purpose 2 ml stock solution was transferred to 20 mlvolumetric flask and volume to the mark with diluent. Systemsuitability was determined by injecting five replicate standard solutionfrom same vial before analyze test sample each day. According toICH guideline the acceptance criteria for system suitability were: relative standard deviation should be less than 2, theoretical plates should be greater than 2000 and tailing factor should be less than 2.(Fig.3).

Table. 1: System suitability studies result of Lafutidine and Domperidone.

Property	Lafutidine	Domperidone
Retention time (t_R)	4.224 (≥ 1)	5.079 (≥ 1)
Tailing factor(T)	1.510	1.630
Therapeutic plate (N)	2092	5239

Specificity: Specificity of an analytical method means to show that the methodwas not affected by the presence of impurities or excipients or and withdiluents.

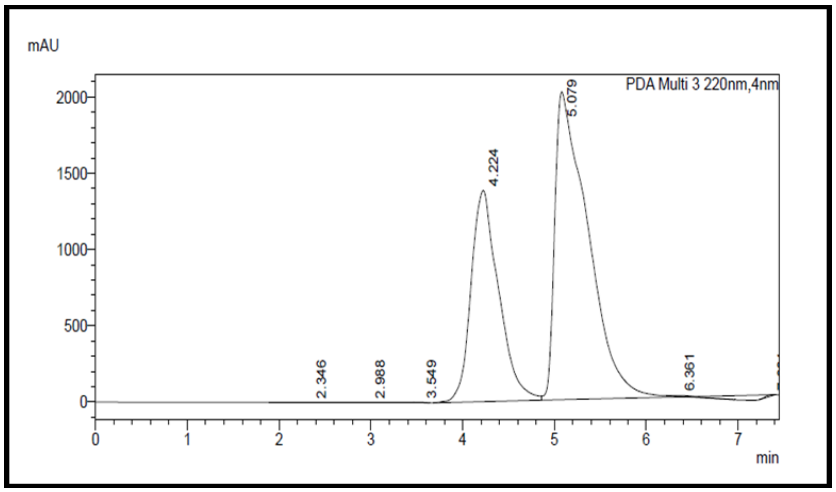

Fig. 3. Chromatogram of Lafutidine and Domperidone.

Table. 2: Observations result of chromatogram Lafutidine and Domperidone.

Peak name	Retention time	Area	Area %	Height	Height %	Asymmetry	Theoretical plate (USP)	Resolution (USP)
Lafutidine	4.224	96.6371	33.02	78194	44.82	1.51	2092	0.00
Domperidone	5.079	197.7143	66.98	96177	55.18	1.63	5239	2.670
Total		294.3514	100.00		100.00			

Linearity and range

Preparation of Calibration graph: In this progression, the aliquots of stock solution of Lafutidine (0.7-1.2mL of 500 µ g mL-1) and Domperidone (0.7-1.2mL of 1500 µ g mL-1)

were transferred into a 10 ml volumetric flasks and made up to the mark with mobile phase, and the solutions contains 35, 40, 45, 50, 55 and 60µ g mL-1 of Lafutidine and 105, 120, 135, 150, 165 and 180 µ g mL- 1 Domperidone. The solutions were injected and the chromatograms were recorded at 220 nm. The above concentration range was found to be linear and obeys Beer's law. The procedure was repeated for six times. The peak areas were plotted against concentration and the calibration curve was constructed.

Table. 3: Calibration data result of Lafutidine and Domperidone.

Sr. No.	Conc. of Lafutidine (µ g mL-1)	Peak area (Response)	Conc. of Domperidone (µ g mL-1)	Peak area (Response)
1.	35	66.5433	105	137.0444
2.	40	75.9021	120	156.025
3.	45	84.9834	135	175.9088
4.	50	94.6271	150	196.7133
5.	55	103.7244	165	216.1200
6.	60	113.4119	180	237.6688

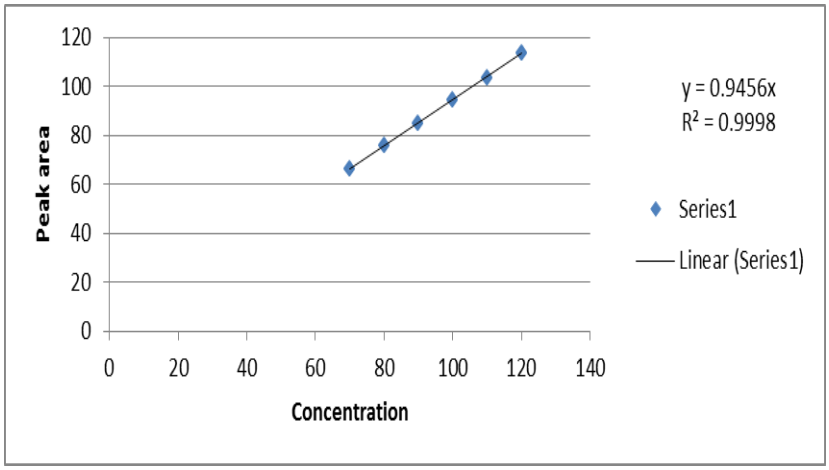

Fig. 4. Calibration curve of Lafutidine (Peak area v/s Conc.).

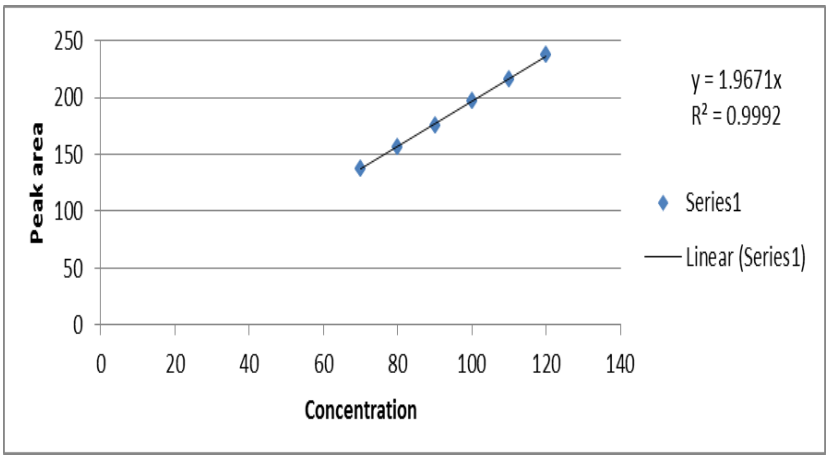

Fig. 5. Calibration curve of Domperidone (Peak area v/s Conc.).

Table. 4: Assay validation sheetresult of Lafutidine and Domperidone.

Sr. No.	Parameter	Lafutidine	Domperidone
1.	Detection wavelength	220nm	220nm
2.	Regression equation	y= 0.9456x	y= 1.9671x
3.	Accuracy	100.9891±2.3144	99.8473±0.5214
4.	Slope	0.9456	1.9671
5.	Intercept	1	1
6.	Linearity range	35-60µ g mL-1	105-180 µ g mL-1
7.	Correlation coefficient	0.999	0.999
8.	SE of Intercept	0.38868	1.3867
9.	SD of intercept	0.95206	3.3968
10.	LOD	3.32257	5.6985
11.	LOQ	10.0684	17.2684

Precision (intraday/ inter-day precision)

Precision (intraday) expressed as an absolute or relative standard deviation(RSD) and does not relate to reference values or actual value. On theother side intermediate precision (inter-day precision) expressed to determination of RSD ofreplicate sample within laboratories variations: different days, different analysts, different column, different HPLC etc. Precision and intermediate precision solution were prepared fromstock solution same as system suitability solution preparation andconcentration was (40 µ g mL-1) and (120 µ g mL-1) of Lafutidine and Domperidone respectively. Precision and intermediate precision testwere done by injecting six replicate standard solutions.

Table. 5: Intraday/ inter-day precision results of Lafutidine and Domperidone.

Sr. No.	Lafutidine (40 µg mL-1)	Domperidone (120 µg mL-1)	Lafutidine (40 µg mL-1)	Domperidone (120 µg mL-1)
1.	75.9521	156.095	75.9521	156.025
2.	75.919	157.145	76.9089	157.126
3.	75.9432	154.188	75.9432	154.105
4.	74.9586	156.104	74.9588	156.104
5.	76.9532	155.185	75.9532	157.528
6.	76.9580	155.184	77.9497	156.052
Mean	76.1140	155.6502	76.2776	156.156
SD	0.7543	0.9318	1.2534	1.1883
% RSD	0.9911	0.5986	1.3442	0.7609

Accuracy: To the formulation (pre-analyzed sample), the reference standards ofthe drugs were added at the level of 80%, 100%, 120%, the method wasdetermined by recovery studies.

Table. 6: Accuracy data result of Lafutidine and Domperidone.

Level	Amount added (µg mL-1)		Amount recovered (µg mL-1)		Recovered %		Result % RSD	
	LAF	DOM	LAF	DOM	LAF	DOM	LOF	DOM
80%	40	120	41.3	121.3	103.2%	101.08%	0.585	0.215
100%	50	150	52	152	104%	101.3%	0.635	0.198
120%	60	180	62.7	182.7	104.5%	101.05%	0.605	0.207

Robustness: Robustness of an analytical procedure has been defined by theInternational Conference on Harmonization (ICH) as a measure of itscapacity to remain unaffected by small, but deliberate variations inmethod parameters. For the determination of a methodsrobustness, many method parameters, such as pH, flow rate, columntemperature, column oven temperature and wavelength etc. If the influence of the parameter was within acceptance range, the parameter was said to be robust. Robustness of the method was carried out by deliberately makingvariation in the flow rate (± 2%) and changing wavelength (± 1). During performing robustness test standardstock solution at concentration (40 µ g mL-1) and (120 µ g mL-1) of Lafutidine and Domperidone in combination was used and it was found that all the criteria for system suitability wassatisfactory. So that it can be concluded that this method was robust atthat changing parameter.

Table. 7: Robustness data result of Lafutidine and Domperidone.

Sr. No.	Robustness condition	Lafutidine % RSD	Domperidone % RSD
1.	Flow rate minus (2%)	0.0642	0.0794
2.	Flow rate plus (2%)	0.7765	0.7646
3.	Wavelength minus(1nm)	0.0021	0.0031
4.	Wavelength plus (1nm)	0.0571	0.0548

Ruggedness/ (reproducibility): In standardstock solution of Lafutidine(500 µ g mL-1) and Domperidone (1500 µ g mL-1). Pipetting 0.9mL of standard stock solution in to 10mL volumetric flask to acquire (45 µ g mL-1) and (135 µ g mL-1) of Lafutidine and Domperidone respectively. Then ruggedness was performed in different days, different time, different person, and then calculating the %RSD of Lafutidine and Domperidone respectively. So that it can be concluded that this method was reproducible atthat changing different conditions.

Table. 8: Ruggedness data of Lafutidine and Domperidone.

Sr. No	Sample	System	Day	Time	Lafutidine % RSD	Domperidone % RSD
1.	Batch 1	Shimadzu Ultra High-Performance Liquid Chromatography (Nexera X2)	Monday	10 AM	0.0059	0.0036
2.	Batch 2	Shimadzu Ultra High-Performance Liquid Chromatography (Nexera X2)	Tuesday	11 AM	0.6388	0.006

Limit of detection: The LOD for this method was determined to be 3.32257µ g mL-1for LAF and 5.6985µ g mL-1 for DOM respectively.

Limit of quantification: The LOQ for this method wasdetermined to be 10.0684µ g mL-1 for LAF and 17.2684µ g mL-1 for DOM respectively.

Assay: Standard preparations are made from the API and sample preparations are from formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The average % assay was calculated and found to be 100.00% and 100.02% for Lafutidine and Domperidone respectively.

Table. 9: % Assay data result of Lafutidine and Domperidone formulation.

Sr. No.	Lafutidine % Assay	Domperidone % Assay
1.	99.859	100.00
2.	99.851	99.99
3.	100.00	100.00
4.	100.13	99.99
5.	100.24	100.04
6.	99.93	100.10
Mean	100.00	100.02
SD	0.1564	0.0452
% RSD	0.156	0.045

CONCLUSION

A simple, precise and accurate method was developed for the quantitative estimation of LAF and DOM in bulk drug and marketed formulation without any interference from the excipients. Calibration curve was linear over the concentration range of 35-60µ g mL-1 and 105-180µ g mL-1 for Lafutidine and Domperidone respectively. The percentage RSD for

precision and accuracy of the method was found to be less than 2%. The low % RSD values for recovery indicated that the method was found to be accurate.

The validation parameter such as system suitability study, specificity, linearity, range, precision, (intraday/ inter-day precision), accuracy, robustness, ruggedness, limit of detection, limit of quantification, and assay were according to ICH guidelines.

The proposed method is rapid, accurate and sensitive. It makes use for fewer amounts of solvents and change of set of conditions requires a short time. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase makes the method especially suitable for routine laboratory and quality control analysis work. The complete separation of the analytes was accomplished in less than 10 min. Therefore, the developed RP-UHPLC methods were found to be simple, precise and reliable for determination of Lafutidine and Domperidone in simultaneously in pharmaceutical formulation.

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