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Analytical Technique Development and Validation for The Simultaneous Estimation of Loratadine and Famotidine in Synthetic Mixture

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ABSTRACT:
Loratadine, a second-generation antihistamine, and famotidine, an H_2-receptor antagonist, are widely used for the management of allergic and gastric acid-related disorders. Recent clinical evidence, including Phase III COVID-19 studies, suggests that their combination is safe, effective, and may exhibit synergistic therapeutic benefits, with additional potential in managing urinary frequency in postmenopausal women. In the present study, simple, precise, and accurate analytical methods were developed and validated using first-order derivative UV spectrophotometry and RP-HPLC for the simultaneous estimation of loratadine and famotidine in a synthetic mixture. For the UV spectrophotometric method, Methanol was used as the solvent for analysis. The first-order derivative UV method was employed using 287 nm and 310 nm wavelength for the quantitative determination of Loratadine and Famotidine, respectively. The zero-crossing point (ZCP) of Loratadine was found to be 310 nm and 287 nm for Famotidine. Chromatographic separation was carried out under isocratic conditions using a C_{18} column. The optimized mobile phase consisted of Phosphate Buffer (pH 3.1): Acetonitrile (68:32 % v/v) to achieve well-resolved and symmetrical peaks of both analytes. The flow rate was maintained at 1.0 mL/min, and detection was performed at 230 nm wavelength. The retention time was found to be 2.5 min for Loratadine and 6.5 min for Famotidine. The linearity of Loratadine and Famotidine for both methods was established in the range of 1-5 µg/mL and 8-40 µg/mL, respectively. The developed methods were validated for parameters including specificity, linearity, range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), assay, and system suitability. All validation results were found to be within the acceptable limits specified by the ICH Q2 (R2) guideline. The proposed methods were linear, sensitive, precise, accurate, and reproducible, and were suitable for routine quality control analysis and quantitative determination of Loratadine and Famotidine in synthetic mixture.

1. INTRODUCTION:

An allergy is a condition in which the immune system reacts abnormally to substances that are usually harmless to most people. These substances, known as allergens, include pollen, dust, pet dander, certain foods, and insect venom. When a person with allergies is exposed to an allergen, their immune system produces antibodies that mistakenly identify it as harmful. This reaction can lead to inflammation affecting different parts of the body such as the skin, sinuses, airways, or digestive system [1]. The severity of allergic reactions varies widely, ranging from mild symptoms like sneezing or itching to severe and potentially life-threatening conditions such as anaphylaxis. Although allergies cannot always be completely cured, various treatments and preventive measures can help manage and reduce their symptoms effectively [1]. Loratadine, a second-generation antihistamine, is primarily used to relieve symptoms of hay fever and other allergies by selectively blocking H1 receptors [2], whereas Famotidine, an H2-receptor antagonist, reduces gastric acid secretion and is widely used in the treatment of gastric ulcers, gastroesophageal reflux disease, and erosive esophagitis [3-4]. Loratadine and Famotidine have been reported as an effective and safe combination therapy for the management of allergic conditions, gastric acid-related disorders, and certain emerging therapeutic conditions such as COVID-19 and urinary bladder dysfunction. Recent clinical studies, including phase 3 trial, have demonstrated that the combination of Loratadine and Famotidine provides synergistic therapeutic effects, improving clinical outcomes in COVID-19 patients and showing promising results in managing urinary frequency in female patients with bladder function disorders. The complementary mechanism of action of these drugs enhances their overall efficacy while maintaining safety and tolerability [5]. A comprehensive literature survey reveals that various analytical techniques for Loratadine including UV spectrophotometry [6, 7], RP-HPLC-UV [8, 9], HPLC [10, 11], Stability indicating RP-HPLC [12, 13], Liquid Chromatographic Method for the Determination of Loratadine and its Impurities [14], Loratadine and its Metabolite Desloratadine in Human Plasma [15, 16] and Loratadine in Human Plasma by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) [17]. Likewise, Famotidine has been quantified using UV-Visible spectrophotometry [18-20], Oral Suspension by RP-HPLC Method [21], Impurity profiling of Famotidine in bulk drugs and pharmaceutical formulations by RP-HPLC method [22], Simultaneous Estimation of Chlorzoxazone, Paracetamol, Famotidine and Diclofenac Potassium in Their Combined Dosage Form by Thin Layer Chromatography [23], have been reported for the individual and with another drug. The literature survey indicates that, despite the availability of several analytical methods for the individual estimation of these drugs, no method has yet been reported for the simultaneous estimation of Loratadine and Famotidine in a synthetic mixture. Therefore, the present study is aimed at the development and validation of simple, accurate, precise, and reproducible UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Loratadine and Famotidine in a synthetic mixture in accordance with ICH Q2 (R2) guideline [24].

2. MATERIALS AND METHODS:

2.1 Compounds and Components:

Loratadine and Famotidine were bought from Dharma Pharma from Ahmedabad as gift samples. Finar Chemicals, Ahmedabad, provided the HPLC-grade methanol, acetone, and water that were employed. Ortho-phosphoric acid (75%, AR grade) and potassium dihydrogen phosphate were procured from Astron Chemicals Ltd., India. All solutions were freshly prepared each day.

2.2 Scientific conditions with instrumentation:

The RP-HPLC analysis was performed using a Systronics LC-138 system integrated with Clarify® software, an SPD-20A UV detector, and a Rheodyne injector featuring a 20 µL sample loop. Chromatographic separation was executed using a reversed-phase strategy. Isocratic elution of both analytes was achieved utilizing a mobile phase consisting of Phosphate Buffer (pH 3.1): Acetonitrile (68:32 % v/v) at a flow rate of 1.0 mL/min. Detection was maintained at an optimal wavelength of 230 nm. Mobile phases were prepared daily, filtered through 0.45 µm Millipore membrane filters, and degassed via a digital ultrasonicator PRO+ 10A (India) prior to use. A Kromstar® C_{18} (250 × 4.6 mm, 5 µm) column was employed. The LC system was operated at ambient temperature. For the UV spectrophotometric method, a Shimadzu 1900 double-beam spectrophotometer with UV Probe 2.7 software and 1.0 cm quartz cells was utilized. Analytical weighing was conducted on a Scale-tec electronic precision balance.

2.3 *Preparation of stock solution:*

Accurately weighed 10 mg each of Loratadine and Famotidine were separately transferred into two 100 ml volumetric flasks. Both drugs were dissolved in methanol and the solutions were diluted up to the mark with the same solvent to obtain standard stock solutions of concentration 100 µg/ml for each drug. These prepared stock

solutions were used for further analytical studies.

2.4 Preparation of standard solution:
0.2 ml from Loratadine stock solution and 1.6 ml from Famotidine stock solution were accurately pipetted into separate 10 ml volumetric flask and diluted up to the mark with methanol to obtain final concentrations of 2 µg/ml and 16 µg/ml, respectively.

2.5 Preparation of standard working solution:
The concentration range of **1-5 µg/mL for Loratadine** and **8-40 µg/mL for Famotidine** were prepared from their respective stock solutions. Aliquots of **Loratadine (0.1, 0.2, 0.3, 0.4, and 0.5 mL)** and **Famotidine (0.8, 1.6, 2.4, 3.2, and 4.0 mL)** were pipetted into 10 different 10 mL volumetric flasks and diluted to the mark with Methanol to obtain final concentrations of **1, 2, 3, 4, and 5 µg/mL for Loratadine** and **8, 16, 24, 32, and 40 µg/mL for Famotidine**, respectively. Under the optimized spectrophotometric conditions, the samples were analyzed using a 1 cm quartz cuvette in the UV spectrophotometer. Similarly, under the optimized RP-HPLC conditions, **20 µL of each standard working solution** was injected into the system for chromatographic analysis.

2.6 Preparation of 10% Orthophosphoric acid:
A 10% orthophosphoric acid solution was prepared by accurately transferring 1.0 ml of concentrated orthophosphoric acid into a 10 ml volumetric flask containing a small quantity of HPLC-grade water. The solution was then diluted up to the mark with HPLC-grade water and mixed thoroughly to obtain the required concentration.

2.7 Preparation of 10mM Phosphate Buffer:
Accurately weighed 0.272 g of potassium dihydrogen phosphate (KH_2PO_4) was transferred into a suitable volumetric flask containing 200 ml of HPLC-grade water and allowed to dissolve completely. The solution was then filtered through a 0.45 µm membrane filter and sonicated for approximately 10 minutes to remove any dissolved gases. The pH of the buffer solution was adjusted to 3.1 using 10% orthophosphoric acid.

3. ANALYTICAL TECHNIQUES:

3.1 Method development:

3.1.1 Method I: UV Spectrophotometric Method:
A first-order derivative spectrophotometric method was employed for the simultaneous estimation of Loratadine and Famotidine in a synthetic mixture. Separate working standard solutions were scanned in the 200-400 nm range to obtain derivative spectra and determine suitable zero-crossing wavelengths for analysis. Standard stock solutions of both drugs were prepared in methanol at 100 µg/mL. Aliquots of 0.2 mL of Loratadine and 1.6 mL of Famotidine were transferred into separate 10 mL volumetric flasks and diluted to the mark with methanol to obtain final concentrations of 2 µg/mL for Loratadine and 16 µg/mL for Famotidine, respectively. The zero-order spectra were recorded and converted to first-order derivative spectra. Upon overlaying, Loratadine showed a zero-crossing at 287 nm, while Famotidine showed a zero-crossing at 310 nm. Quantification was performed at 287 nm for Loratadine (ZCP of Famotidine) and 310 nm for Famotidine (ZCP of Loratadine). The zero- and first-order overlain spectra are presented in Figure 1 and 2, respectively.

3.1.2 Method II: Reverse Phase High Performance Liquid Chromatography:
The isocratic analysis was performed using a reverse-phase chromatographic technique, which is suitable for moderately polar to non-polar ionic compounds. Various mobile phase compositions, including Methanol: Water, Acetonitrile: Water, and Acetonitrile: Phosphate Buffer (pH 4), were initially tested in different proportions. The combination of Phosphate Buffer (pH 3.1 adjusted with 10% ortho-phosphoric acid) and Acetonitrile (68:32 % v/v) provided optimal polarity, allowing proper migration, separation, and resolution of Loratadine and Famotidine. Under these conditions, the eluted peaks were well defined and completely resolved. Prior to use, all solvents were filtered through a 0.45 µm membrane filter and sonicated for 10 minutes. The stationary phase consisted of a Kromstar C_{18} column (250 mm × 4.6 mm, 5 µm), and the eluent was monitored using a UV detector at 230 nm, as shown in Figure 1.

3.2 Method Validation:
According to ICH guideline Q2 (R2) [24], the approaches have been verified and confirmed. The technique has undergone rigorous validation through a variety of evaluations for System suitability, Specificity, Linearity and

range, Accuracy, Precision, Limit of detection, and Limit of quantification.

3.2.1 Specificity:
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradation products, and excipients. For Loratadine and Famotidine, specificity was demonstrated by comparing the chromatogram of the mobile phase and the test preparation solution, which confirmed that there was no interference from the mobile phase or excipient peaks with the peaks of Loratadine and Famotidine.

3.2.2 Linearity and Range (n=6):
Loratadine and Famotidine exhibited good linearity within the concentration ranges of 1-5 µg/mL and 8-40 µg/mL, respectively. In the UV spectrophotometric method, calibration graphs were generated by plotting absorbance against concentration (µg/mL). For the HPLC method, calibration plots were constructed by correlating peak area with the respective concentrations of Loratadine and Famotidine. Linearity of both the drugs was checked in terms of slope, intercept and correlation coefficient.

3.2.3 Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, reproducibility (Interday precision), and repeatability.

3.2.3.1 Intraday Precision (n=3): Solutions containing 1, 2, 3 µg/mL of Loratadine and 8, 16, 24 µg/mL of Famotidine were analyzed three times on the same day, and % R.S.D. was calculated.

3.2.3.2 Interday Precision (n=3): Solutions containing 1, 2, 3 µg/mL of Loratadine and 8, 16, 24 µg/mL of Famotidine were analyzed on three different successive days, and % R.S.D. was calculated.

3.2.3.3 Repeatability (n=6): Solutions containing 2 µg/mL of Loratadine and 16 µg/mL of Famotidine were analyzed six times, and % R.S.D. was calculated.

3.2.4 Limit of Detection (LOD):
The limit of detection for Loratadine and Famotidine was determined according to ICH guidelines using the following equation:
$LOD = 3.3 \times (\sigma / S)$
Where,
σ = standard deviation of the Y intercept of the calibration curve
S = slope of the calibration curve.

3.2.5 Limit of Quantification (LOQ):
The limit of quantification can be calculated according to ICH guidelines using the following equation:
$LOQ = 10 \times \sigma/s$
Where,
σ = standard deviation of the Y-intercept of the calibration curve.
S = Mean slope of the corresponding calibration curve.

3.2.6. Accuracy:
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted as either a conventional true value or an accepted reference value, and the value found. Accuracy of the developed method was confirmed by performing a recovery study as per ICH guideline at three different concentration levels 50%, 100%, and 150%, and the values were measured for Loratadine (2 µg/ml) and Famotidine (16 µg/ml). This assessment was carried out in triplicate to ensure the reliability and reproducibility of the results.

3.2.7 Robustness:
Robustness refers to the ability of an analytical method to produce consistent and reliable results despite small,

intentional changes in experimental conditions. It demonstrates the method's dependability during routine use. In liquid chromatography, robustness is evaluated by slightly varying parameters such as mobile phase pH, composition, column type, batch, temperature, and flow rate. If these minor modifications do not significantly affect results like retention time, resolution, or assay values, the method is considered robust.

3.2.9 System suitability test:

A system suitability test is a crucial component of liquid chromatography, performed to ensure that the chromatographic system is functioning properly before analysis. It confirms that the system provides adequate resolution and consistent reproducibility for accurate results. Key parameters evaluated in this test include resolution, column efficiency, tailing factor, and the number of theoretical plates, all of which collectively indicate whether the system's performance meets the required standards for reliable analysis.

3.2.10 Assay:

A synthetic mixture of Loratadine and Famotidine was prepared in a 1:8 ratio along with common excipients and mixed thoroughly. Common excipients such as Microcrystalline cellulose (7 mg), Lactose (72 mg), Talc (22 mg), Magnesium Stearate (5 mg), and Croscarmellose sodium (4 mg) were added in a mortar and pestle along with Loratadine (10 mg) and Famotidine (80 mg). This mixture was transferred to a 100 ml volumetric flask, sonicated, and diluted to volume with methanol. The solution was filtered through Whatman filter paper, yielding final concentrations of 100 µg/ml and 800 µg/ml, respectively. The filtrate was further diluted to the mark with methanol. The final working concentrations of Loratadine and Famotidine were 2 µg/ml and 16 µg/ml, respectively, for which 0.2 ml of the combined solution was scooped out into a 10 ml volumetric flask and diluted appropriately with methanol. This solution was injected into UV Spectrophotometer (Shimadzu 1900) and RP-HPLC for calculated % assay from its Absorbance and Peak area with the help of regression equation, respectively.

4. RESULTS AND DISCUSSION:

4.1 Method I: UV Method:

Among various UV-spectrophotometric techniques, the first-order derivative method offers distinct advantages over conventional zero-order UV methods for the simultaneous estimation of multiple components. This technique enhances spectral resolution by minimizing spectral overlap and background interference, thereby improving selectivity and accuracy. Unlike the simultaneous estimation and absorbance ratio methods, first-order derivative spectrophotometry allows quantification at zero-crossing wavelengths, enabling precise estimation of each component without mutual interference. Additionally, the method demonstrates improved sensitivity, better baseline correction, and reduced matrix effects. Owing to its simplicity, rapid analysis, and ability to resolve overlapping spectra, first-order derivative UV spectrophotometry is highly beneficial for the routine analysis of combined pharmaceutical formulations, Loratadine and Famotidine.

4.1.1 Selection of detection wavelength for Loratadine and Famotidine:

The remarkable absorbance of Loratadine exhibited an absorption maximum at 248 nm, while Famotidine showed an absorption maximum at 287 nm, as shown in Figure 1. The zero-order and first-order UV absorption spectra of Loratadine (2 µg/ml) and Famotidine (16 µg/ml) in Methanol are presented in Figure 1 and 2, respectively.

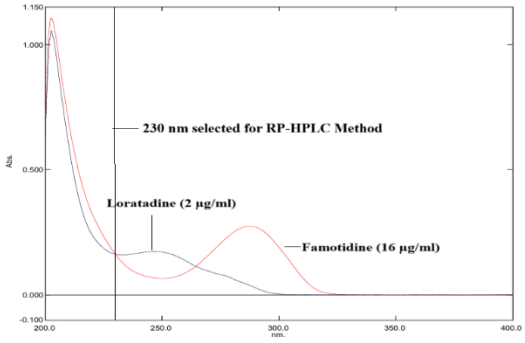

Figure 1: Overlain Zero Order UV Spectra of Loratadine (16 µg/ml) and Famotidine (16 µg/ml) in Methanol

4.1.2 First-order derivative UV Method Development:

The overlapping absorption of Loratadine and Famotidine in the 200–400 nm range is evident from the spectra, which makes direct quantification by conventional UV spectrophotometry difficult without compensating for spectral interference. The total absorbance of a mixture at a specific wavelength represents the sum of the

individual absorbances of both drugs. When the absorption bands overlap, the concentration of each drug can be determined using its zero-order spectra [Figure 1]. To eliminate interference from overlapping components, the absorption spectra were converted into first-derivative spectra using $\Delta\lambda = 2$ nm and a scaling factor of 4. The amplitude values were measured at 287 nm (λ_1) for Loratadine and at 310 nm (λ_2) for Famotidine, as shown in Figure 2.

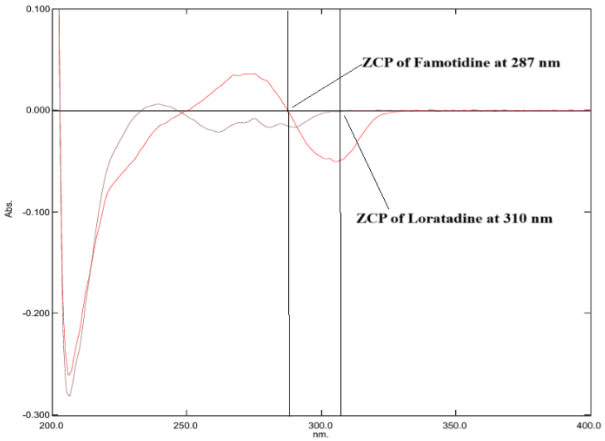

Figure 2: Overlain First order UV Spectra of Loratadine (16 µg/ml) and Famotidine (16 µg/ml) in Methanol

4.2 Method II: RP-HPLC Method:

Pharmaceutical analysis commonly uses simultaneous estimation using RP-HPLC. It enables the use of RP-HPLC to determine the presence of many chemicals in a sample. For the simultaneous estimate of various components, including medications and their contaminants, in pharmaceutical formulations, a number of techniques have been devised and proven effective. Utilizing an appropriate column, mobile phase, and detection equipment, the simultaneous estimation technique by HPLC allows for the separation and quantification of the target substances. In pharmaceutical analysis, reverse-phase high-performance liquid chromatography (RP-HPLC) is a great instrument for simultaneous estimation that offers confidence and specificity for the identification of chemical entities in a synthetic mixture. Reverse-phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient, but also performs better in terms of efficiency, stability and reproducibility. The C_{18} column was selected because it is the least polar compared to the C_4 and C_8 columns. The C_{18} column allows eluting polar compounds more quickly compared to non-polar compounds. In addition to this, a UV detector is used, which allows easy detection of the compounds in UV-transparent organic solvents. Hence, a C_{18} (250×4.6 mm) column of 5 µm particle packing was selected for the separation of Loratadine and Famotidine.

4.2.1 Selection of detection wavelength:

The sensitivity of the RP-HPLC method that uses UV detection depends upon the proper selection of the detection wavelength. At 230 nm, both drugs give good peak height and shape. So, 230 nm was selected for the simultaneous estimation of Azithromycin Dihydrate and Metronidazole Benzoate in a synthetic mixture. Overlay UV spectra of Loratadine (2 µg/ml) and Famotidine (16 µg/ml) in Methanol are shown in Figure 1.

4.2.2 RP-HPLC Method Development:

Liquid chromatography coupled with UV detection was used to develop a way for simultaneously measuring Loratadine and Famotidine. Achieving acceptable peak symmetry and theoretical plates within a realistic time period was the aim. The chromatographic conditions were optimized by experimenting with various stationary and mobile phases. The mobile phase Phosphate Buffer (pH 3.1 adjusted with 10% ortho phosphoric acid): Acetonitrile (68:32 %v/v) was selected because it was found to ideally resolve the peaks with retention times of 2.5 min and 6.5 min for Loratadine and Famotidine, respectively, shown in Table 1. A Kromstar C_{18} (250×4.6 mm, 5 µm) column was used for the separation of Loratadine and Famotidine with a flow rate of 1.0 ml/min at 230 nm.

Table 1: System suitability parameters

Parameters	Retention time	Tailing Factor	Number of Theoretical Plates	Resolution

Loratadine	2.5min	0.82	6765	4.0
Famotidine	6.5min	1.12	5972	

4.3 Validation of the proposed methods:

4.3.1 Linearity and range:

For the UV method, the mean absorbance was measured for the linearity of Loratadine (1-5 µg/ml) at 287 nm and Famotidine (8-40 µg/ml) at 310 nm, shown in Figures 3 and 4, respectively.

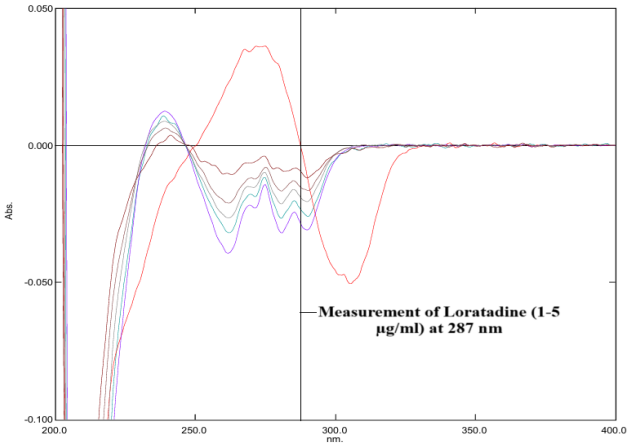

Figure 3: Overlain UV spectra of (a) Loratadine (1-5 µg/ml) at 287 nm

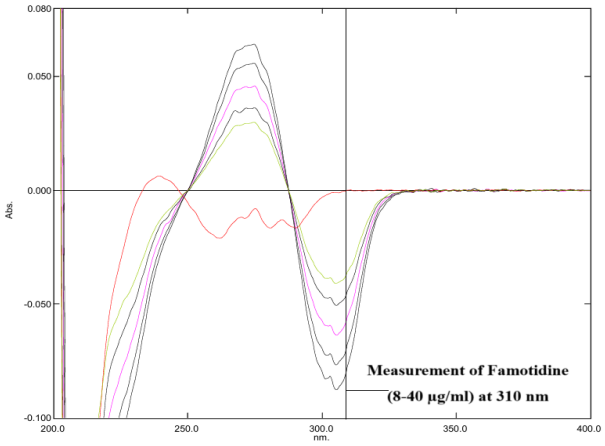

Figure 4: Overlain UV spectra of Famotidine (8-40 µg/ml) at 310 nm

The linearity data of the UV method showed in Table 2.

Table 2: Results of validation parameters for UV and RP-HPLC method

Sr No.	Validation Parameters	RP-HPLC Method		UV Method	
		Loratadine	**Famotidine**	**Loratadine**	**Famotidine**
1.	Wavelength (nm)	230 nm		287nm	310nm
2.	Linearity and Range	1-5	8-40	1-5	8-40
3.	Regression equation (y = mx + c)	y = 238.56x – 144.37	y = 84.42x – 294.94	y = 0.0045x + 0.0043	y = 0.0012x + 0.0259
4.	Correlation Coefficient (r^2)	0.999	0.9983	0.9985	0.997
5.	Intraday Precision (% RSD, n=3)	0.65-1.13	0.34-1.03	0.94-1.17	0.61-1.22
6.	Interday Precision (%RSD, n=3)	0.66-1.17	0.36-1.04	0.97-1.20	0.65-1.25
7.	Repeatability (% RSD, n=6)	0.72	0.64	1.06	0.95
8.	Accuracy (% Recovery, n=3)	99.66%-99.90%	99.91%-99.98%	99%-99.87%	99.79%-99.95%

9.	LOD (µg/ml)	0.02	0.16	0.08	1.18
10.	LOQ (µg/ml)	0.05	0.49	0.24	3.58
11.	%Assay	99.85%	99.96%	99.85%	99.87%

For the RP-HPLC method, the linearity of Loratadine and Famotidine was found to be 1-5 µg/ml and 8-40 µg/ml at 230 nm, respectively. Linearity data for the RP-HPLC method showed in Table 2.

4.3.2 Precision:

Precision was assessed by intraday, interday, and repeatability studies. Loratadine (1,3,5 µg/ml) and Famotidine (8,16,24 µg/ml) were analyzed in triplicate on the same day and on three consecutive days. Repeatability was evaluated at 2 µg/ml for Loratadine and 16 µg/ml for Famotidine. The %RSD values for intraday, interday, and repeatability were all less than 2%, as shown in Table 2.

4.3.3 LOD and LOQ:

The limits of detection (LOD) for the UV method were found to be 0.08 µg/mL for Loratadine and 1.18 µg/mL for Famotidine, while the corresponding limits of quantification (LOQ) were 0.24 µg/mL for Loratadine and 3.58 µg/mL for Famotidine, respectively. In comparison, the RP-HPLC method exhibited lower LOD values of 0.02 µg/mL for Loratadine and 0.16 µg/mL for Famotidine, with LOQ values of 0.05 µg/mL and 0.49 µg/mL, respectively, indicating higher sensitivity of the HPLC method. The results of LOD and LOQ for both methods are shown in Table 2.

4.3.4 Accuracy:

The accuracy of the method was evaluated by recovery studies using the standard addition method. Known amounts of Loratadine and Famotidine were added to the pre-analysed sample at 50%, 100%, and 150% levels. The studies were performed in triplicate, and accuracy was expressed as % recovery. For the UV method, Loratadine showed mean recoveries ranging from 99.00% to 99.87%, while Famotidine exhibited recoveries between 99.79% and 99.95%. Similarly, the RP-HPLC method demonstrated mean recoveries of 99.66% to 99.90% for Loratadine and 99.91% to 99.98% for Famotidine. These results (Table 3) confirmed the high accuracy and reliability of both analytical methods.

Table 3: Recovery study data for UV and RP-HPLC Method

Name of Drug	% Level of recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Std Amt (µg/ml)	Total amount Recovered (µg/ml)	% Mean Recovery ± SD(n=3)
UV-Method						
Loratadine	50	2	1	3	2.97	99±0.509
	100	2	2	4	3.96	99.17±0.381
	150	2	3	5	4.99	99.87±0.305
Famotidine	50	16	8	24	23.95	99.79±0.283
	100	16	16	32	31.97	99.92±0.347
	150	16	24	40	39.98	99.95±0.425
RP-HPLC Method						
Loratadine	50	2	1	3	2.99	99.66±0.2161
	100	2	2	4	3.99	99.75±0.3252
	150	2	3	5	4.995	99.90±0.4343
Famotidine	50	16	8	24	23.98	99.91±0.1434
	100	16	16	32	31.99	99.96±0.2525
	150	16	24	40	39.995	99.98±0.3616

4.3.5 Assay as Analysis of Synthetic Mixture:

From the assay, the final concentration of Loratadine was 2 µg/ml and Famotidine 16 µg/ml were run into UV and HPLC. Assay of the synthetic mixture by the UV method yielded assay values of 99.85 ± 0.015% for Loratadine and 99.87 ± 0.011% for Famotidine (n = 3). Similarly, the RP-HPLC method showed assay values of 99.85 ± 0.136% and 99.96 ± 0.547% for Loratadine and Famotidine, respectively, confirming the accuracy and precision of both methods. Its results are shown in Table 4.

Table 4: Analysis of synthetic mixture for UV and RP-HPLC Method

Name of Drug	Amount in synthetic mixture (µg/ml)	Mean Amount found (µg/ml)	% Assay ± SD (n=3)	%RSD
UV-Method				
Loratadine	2	1.97	99.85±0.015	0.016

Famotidine	16	15.98	99.87±0.011	0.012
RP-HPLC Method				
Loratadine	2	1.997	99.85±0.136	0.136
Famotidine	16	15.995	99.96±0.547	0.547

4.3.6 Robustness:

The robustness of the developed HPLC method was assessed by introducing small, intentional variations such as changes in analyst, slight modifications in flow rate, run time, and detection wavelength. The %RSD values were found to be within acceptable limits, confirming the method's robustness and reproducibility (Table 5).

Table 5: Robustness data

Condition	**Variation**	**Loratadine**	**Famotidine**
		% Assay ± SD (n=3)	**% Assay ± SD (n=3)**
Flow rate (1 ml±0.2 ml/min)	0.8 ml/min	99.74±1.12	99.25±9.45
	1.0 ml/min	99.99±1.42	99.58±0.55
	1.2 ml/min	98.99±1.84	99.65±3.76
Detection wavelength (230 nm±2 nm)	228	99.87±1.07	98.94±1.11
	230	99.95±1.42	99.94±0.55
	232	99.75±1.84	99.97±1.18
Mobile Phase Phosphate Buffer (pH 3.1): ACN (68:32 ±2 %v/v)	66:34	99.41±4.51	98.95±2.37
	68:32	99.98±5.42	99.86±0.55
	70:30	99.65±6.47	99.55±1.02

5. CONCLUSION:

The present study successfully demonstrated the development, optimization, and validation of first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Loratadine and Famotidine in a synthetic mixture. The first-order derivative UV method effectively resolved overlapping spectra by employing zero-crossing wavelengths, allowing selective, accurate, and rapid quantification of both analytes. The RP-HPLC method provided efficient chromatographic separation with well-resolved peaks, acceptable retention times, good peak symmetry, and satisfactory system suitability parameters. Both analytical methods were validated in accordance with ICH Q2(R1) and Q2(R2) guidelines. Validation results confirmed excellent linearity within the studied concentration ranges, high accuracy with recoveries close to 100%, and precision demonstrated by low %RSD values for intraday, interday, and repeatability studies. The LOD and LOQ values indicated adequate sensitivity of both methods, with the RP-HPLC method exhibiting comparatively higher sensitivity. Specificity and robustness studies further confirmed that the methods are reliable and unaffected by minor variations in analytical conditions. Statistical comparison using Student's t-test showed no significant difference between the results obtained by the first-order derivative UV method and the RP-HPLC method for recovery and assay parameters at a 95% confidence level, confirming that both methods were statistically comparable in terms of accuracy and precision. Overall, the developed UV and RP-HPLC methods are simple, precise, accurate, reproducible, and suitable for routine quality control analysis and assay of Loratadine and Famotidine in synthetic mixtures.

6. FUTURE PERSPECTIVES:

The developed first-order derivative UV method can be effectively utilized as a rapid and cost-efficient technique for routine quality control analysis of Loratadine and Famotidine. The RP-HPLC method, owing to its higher sensitivity and specificity, may be further extended for stability studies, impurity profiling, and analysis of finished pharmaceutical dosage forms containing these drugs. Future investigations may also include the application of these methods to marketed formulations and further enhancement toward stability-indicating, bioanalytical, and pharmacokinetic studies.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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