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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IBUPROFEN AND FAMOTIDINE

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ABSTRACT

The main objective of the proposed work is to develop and validate a high precision and accurate analytical method for the simultaneous estimation of Ibuprofen and Famotidine in pharmaceutical dosage form by reverse phase high performance liquid chromatography (RP- HPLC) and validate the method as per ICH guidelines. Solubility determination of Ibuprofen and Famotidine in various solvents and buffers. Determine the absorption maxima of both the drugs UV-VISIBLE region in different solvents/buffers and selecting the solvents for HPLC method development. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1] Initially method development work was started by taking UV-visible spectra from 400-200 nm of Famotidine and Ibuprofen (10ppm) standard solutions. By observing the overlain spectra of standard solutions λmax 231 nm was taken for trials to develop HPLC method. The relationship between the concentration of Famotidine and ibuprofen and area of Famotidine and ibuprofen is linear in the range examined since all points lie in a straight line as shown in Figures 8.21, 8.22 and 8.23 respectively and the correlation coefficient is well within limits.

KEY WORDS

Analytical method, Ibuprofen, Famotidine.

2.1 INTRODUCTION

AIM: The main objective of the proposed work is to develop and validate a high precision and accurate analytical method for the simultaneous estimation of Ibuprofen and Famotidine in pharmaceutical dosage form by reverse phase high performance liquid chromatography (RP- HPLC) and validate the method as per ICH guidelines.

OBJECTIVE: To develop a new analytical method for simultaneous estimation of Ibuprofen and Famotidine in bulk and combination by RP-HPLC and validate the above method as per ICH guidelines.

A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result .it should be simple as possible, yet it should allow the use of sophisticated tools such as computer modeling. [7]

Nature of sample

Before beginning of method development, we need to review about the sample, in order to define goals of separation. The kinds of sample related information that can be important are:

The various kinds of sample related information that may be important for method development was summarized in Table 1.3

Method requirements

The goals of the analytical method that need to be developed are considered. The detection limits, selectivity, linearity, range, accuracy and precision are defined.

Literature search and prior methodology

The information related to the analyte is surveyed for synthesis, physical and chemical properties, solubility and relevant analytical methods. Books, periodicals and USP/NF, and publications are reviewed.

Choosing a method

a. Using the information in the literatures, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for analytes.
b. If there are no prior methods for the analyte in the literature, from analogy, the compounds that are similar in structure and chemical properties are investigated and are worked out. There is usually one compound for which analytical method already exist that is similar to the analyte of interest. [7]

Instrumental setup and initial studies

a) The required instrumentation is setup installation, operational and performance qualifications of instrumentation verified by using laboratory standard operating procedures.

b) Always new solvents, filters are used, for example, method development is never started, on a HPLC column that has been used earlier.

c) The analyte standard in a suitable injection/introduction solution and in known concentrations and solvents are prepared. It is important to start with an authentic, known standard rather than with a complex sample matrix. If the sample is extremely close to the standard (ex: bulk drug) then it is possible to start work with the actual sample.

Optimization

During optimization one parameter is changed at a time and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan and every step is documented (in lab notebook) in case of dead ends. Reversed-Phase HPLC offers multiple parameters for optimizing a separation. To plan separation by RP-HPLC, the analyst must select both a stationary phase and a mobile phase appropriate to the analyte under investigation. In addition, the analyst must identify chromatographic conditions that will maintain the sharpness of analyte bands as the sample moves through the stationary phase column to the detector. The first attempt at optimization of separation requires selection of a promising set of conditions.

To optimize the solvent strength, one approach is to begin with a mobile phase that is probably too strong and reduce solvent strength to increase k' (capacity factor, is the measure of retention) between successive runs. When all the peaks fit within the range 0.5< k' < 20, the mobile phase is near optimum from the standpoint of solvent strength.

Table 2.2: Preferred experimental conditions for the initial HPLC separation. [7]

Separation Variable	Preferred Initial Choice
Column	
Dimensions(length,ID)	15 × 0.46 cm
Particle size	5 µm [a]
Stationary phase	C_8 or C_{18}
Mobile phase	
Solvents A and B	Buffer – acetonitrile
%B	80-100 % [b]
Buffer (compound, P^H, concentration)	25Mmpotassium phosphate, 2.0 < Ph < 3.0 [c]
Additives (eg., amine modifiers, ion pair reagents)	Do not use initially
Flow rate	1.5 -2.0 mL/min
Temperature	35-45 ^{0}C
Sample size	
Volume	<25 µL
Weight	<100 µg

3.5 µm particles are an alternative, using a 7.5 cm column. For an initial isocratic run, an initial gradient run is preferred. No buffer required for neutral samples, for pH <2.5, pH-stable columns recommended.

Once the solvent strength has been properly adjusted for the sample, the next selection parameter that can be explored is α (band spacing). In many cases, it is possible to obtain a satisfactory separation simply by varying solvent strength.

This is usually sufficient for simple and easily resolved samples such as less polar and medium polar non-ionic solutes, but for samples containing ionic and ionizable compounds, apart from changes in solvent strength, several other separation variables are to be altered to optimize band spacing, retention or peak shape. [7]

A temperature of 40-60°c appears to be convenient in reversed phase-LC, when sample component permits. Compared to ambient conditions operation at these temperatures usually doubles the column efficiency in terms of number of theoretical plates. Different concentrations of the various organic solvents are required to maintain constant solvent strength. Varying the pH of mobile phase may drastically alter separation selectively in RP-HPLC, if the sample components are acids or bases. When dealing with a sample component of weak base, pKa 8 the ion suppression technique can be used by adding an acidic buffer. If this is ineffective, an ion pair reagent, such as alkyl sulfonic acid may be added. For strong bases, pKa > 8, ion pairs are more effective than ion suppression.

Documentation of analytical figures of merit

The originally determined analytical figures of merit Limit of Quantitation (LOQ), Limit of Detection (LOD), Linearity, time per analysis, cost, sample preparation etc., are documented.

Evaluation of method development with actual samples

The sample solution should lead to absolute identification of the analyte peak of interest apart from all other matrix components.

RESULTS AND DISCUSSION

ANALYTICAL METHOD DEVELOPMENT:

selection of wavelength

Preparation of standard solution of Famotidine

About 10 mg of Famotidine was weighed and taken in a 10-mL volumetric flask, to this 5 mL of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Further dilution was made by taking 1 mL of the above prepared solution into 10 mL volumetric flask to obtain a concentration of 100 µg/ mL of the drug.

Further dilution was made by taking 1mL of above prepared solution into 10 mL volumetric flask and volume made up to mark to obtain a concentration of 10µg/mL of the drug.

Preparation of standard solution of Ibuprofen

About 10 mg of ibuprofen was weighed and taken in a 10-mL volumetric flask, to this 5 mL of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Further dilution was made by taking 1 mL of the above prepared solution into 10 mL volumetric flask to obtain a concentration of 100 µg/ mL of the drug.

Further dilution was made by taking 1mL of above prepared solution into 10 mL volumetric flask and volume made up to mark to obtain a concentration of 10µg/mL of the drug.

Optimization of UV conditions:

Initially method development work was started by taking UV-visible spectra from 400-200 nm of Famotidine and Ibuprofen (10ppm) standard solutions. By observing the overlain spectra of standard solutions λmax 231 nm was taken for trials to develop HPLC method

METHOD DEVELOPMENT

The objective of this experiment was to optimize the assay method for estimation of Famotidine and Ibuprofen based on the literature survey made, so here the trails mentioned describes how the optimization was done.

Trail: 1

Preparation of Mobile phase

Preparation of Standard Solutions of Famotidine and Ibuprofen

Chromatographic conditions:

Mobile phase: pH 3 buffer: ACN (50:50)

Flow rate: 0.7 mL/min

Column: c18

Detector wavelength: 231 nm

Injection volume: 20 µL

Sl.No	Peak name	RT	Peak area	TP	Rs	Asymmetry
1	Famo	1.865	1351873.250	617.388	-	1.265
2	Ibu	3.432	715705.813	1686.779	4.196	1.250

Trail: 2

Chromatographic conditions:

Mobile phase: phosphate buffer: ACN (65:35)

Flow rate: 0.8 mL/min

Column= c18

Detector wavelength: 231 nm

Injection volume:10 µL

s.no	Peak name	RT	Peak area	TP	Rs	TF
1	unknown	0.207	116262.102	30.325	-	2.138
2	Famotidine	2.123	1479440.750	500.769	6.150	1.069
3	Ibuprofen	3.890	816310.688	1758.607	4.00	1.272

Trail: 3

Chromatographic conditions:

Mobile phase: OPA buffer: ACN (50:50)

Flow rate: 0.6 mL/ min

Column: c18

Detector wavelength: 231 nm

Injection volume: 20 µL

s.no	Peak name	RT	Peak area	TP	Rs	TF
1	Famo	1.815	563276	811.109	-	1.345
2	Ibu	3.707	10893471	1935.017	5.413	1.440

Trail 4

Chromatographic conditions

Mobile phase: buffer (pH 4 adjusted with OPA) : ACN (50:50)

Flow rate: 0.6mL/ min

Column: c18

Detector wavelength: 231 nm

Injection volume: 20 µL

s.no	Peak name	RT	Peak area	TP	Rs	TF
1	unknown	0.240	143445.344	35.358	-	3.810
2	Famo	2.432	621519.813	1253.361	8.539	1.317
3	Ibu	4.615	12317436.0	1965.714	5.369	1.331

Trail 5

Chromatographic conditions:

Mobile phase: buffer (pH 4 adjusted with OPA) : ACN (50:50)

Flow rate: 0.8mL/ min

Column: c18

Detector wavelength: 231 nm

Injection volume: 20 µL

s.no	Peak name	RT	Peak area	TP	Rs	TF
1	Famo	1.915	513246.688	1233.583	-	1.233
2	Ibu	3.173	5867314.5	1893.081	4.194	1.401

Trail: 6

Chromatographic conditions:

Mobile phase: OPA buffer: ACN (50:50)

Flow rate: 0.7 mL/min

Column:c18

Detector wavelength: 231 nm

Column Injection volume: 20 µL

S.NO	PEAK NAME	RT	PEAK AREA	TP	Rs	TF
1	Famo	1.898	268754.594	1466.767	-	1.216
2	Ibu	3.98	3397840.5	1953.432	4.260	1.389

Trail: 7

Chromatographic conditions

Mobile phase: 0.1% Orthophosphoric acid: ACN (50:50)

Flow rate: 0.8 mL/min

Column: c18(X-terra 4.6 mm×150 mm, 5µm)

Detector wavelength: 231 nm

Column Injection volume: 20 µL

s.no	Peak name	RT	Peak area	TP	Rs	TF
1	Famo	2.473	216752.406	4033.222	-	1.186
2	Ibu	3.407	1357775.750	2619.485	3.758	1.127

Optimized Method Parameters:

Table No. 6: Optimized Method Parameters

PARAMETERS	CONDITIONS
Mobile Phase	0.1 % Orthophosphoric acid: Acetonitrile (50:50)
Column (Stationary Phase)	X-terra($C1_8$) (4.6mm x 150mm, 5µm)
Flow rate (ml/min)	0.8 mL/min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20 µL
Detection wavelength (nm)	231 nm
Drug RT (min)	Famotidine 2.473 min Ibuprofen 3.407 min

PROCEDURE:

Preparation of buffer:

Taken 1000ml of HPLC grade water. Added the 1 ml of Orthophosphoric acid.

Preparation of mobile phase

A mixture of above prepared buffer 500 ml (80%), and 500 ml of HPLC grade Acetonitrile (50%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filtered through 0.45 µ filter under vacuum.

ASSAY:

Preparation of the Famotidine and Ibuprofen standard & sample solution:

Preparation of Standard Solution:

Transferred 240 mg of Famotidine and 8 mg of Ibuprofen working standard into a 100ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (2400, 80µg/ml)

From this, 5 ml of the solution was pipette into another 50ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution:

Accurately weighed and transferred tablet powder equivalent to 240 mg of Famotidine and 8 mg of Ibuprofen working standard into a 100ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (2400, 80µg/ml)

From this, 5 ml of the solution was pipette into another 50ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Famotidine and Ibuprofen peaks were measured. %Assay was calculated by using the formulae.

Calculation:

$$\text{ASSAY \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AVG\ WT}{LC} \times 100$$

Where:

AT = Average area counts of sample preparation.

AS = Average area counts of standard preparation.

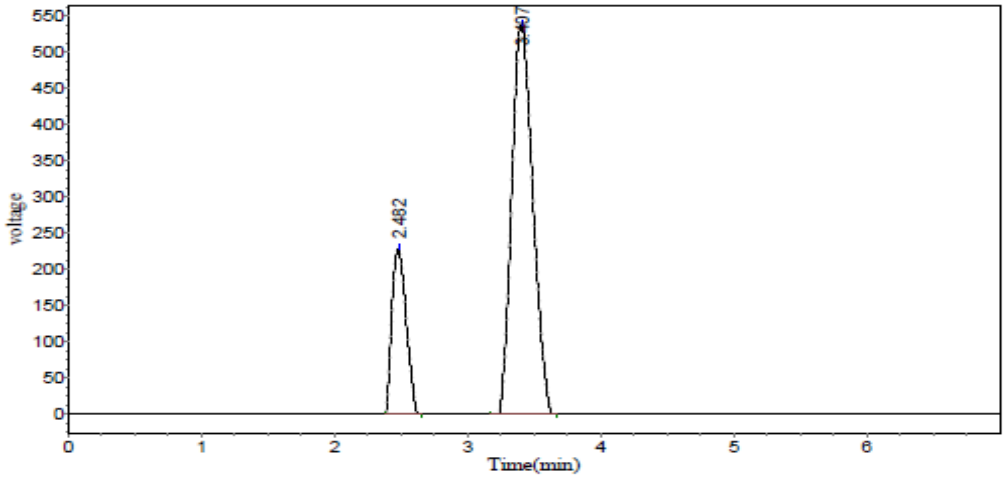
WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = label claim mg/mL

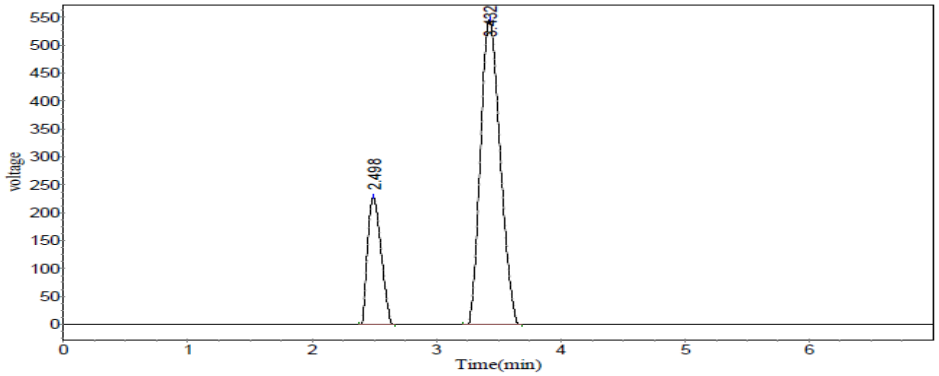
Avg wt = average weight

Standard chromatogram



s.no	Peak name	RT	Peak area	TP	Rs	TF
1	Famotidine	2.473	1701986	2883.618	-	1.275
2	Ibuprofen	3.407	5899512	2959.965	3.066	1.156

Sample chromatogram:



s.no	Peak name	RT	Peak area	TP	Rs	TF
1	Famo	2.498	172220.625	2755	-	1.294
2	Ibu	3.432	5996133.50	2990	3.077	1.169

Drug	Label claim (mg)	Amount found (mg)	% assay
Famotidine	26.6	26.2	99.49
Ibuprofen	800	798.5	98.4

The results of the % Assay calculated are given in Table 8.14 and the values were found to be in limits (98 % - 101%)

ANALYTICAL METHOD VALIDATION

Validation:

Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

A) Accuracy
B) Precision
C) Intermediate Precision
D) Linearity
E) Limit of detection
F) Limit of quantitation
G) Robustness
H) System Suitability

A) ACCURACY:

The closeness of agreement between the true values which is accepted either conventional new value or an accepted reference value and the value found.

Preparation of Sample solution:

Accurately weighed and transferred tablet powder equivalent to 240 mg of Ibuprofen and 8 mg of Famotidine working standard into a 100ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (2400, 80µg/ml)

For preparation of 50% solution (With respect to target Assay concentration):

From this above 2.5 ml of the standard solution and 5 ml of sample solution was pipetted into another 50ml volumetric flask and diluted up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

From this above 5 ml of the standard solution and 5 ml of sample solution was pipetted into another 50ml volumetric flask and diluted up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

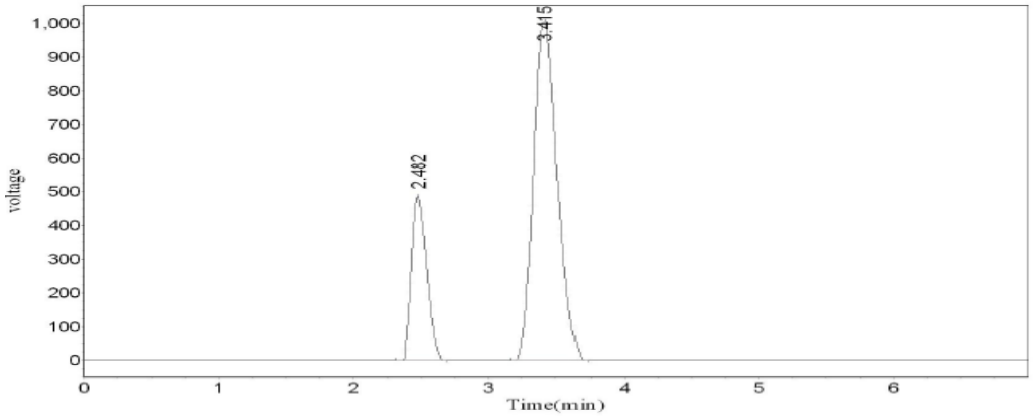
From this above 7.5 ml of the standard solution and 5.0 ml of sample solution was pipetted into another 50ml volumetric flask and diluted up to the mark with diluent.

Procedure:

The standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were injected into the chromatogram.

Sample preparation for accuracy

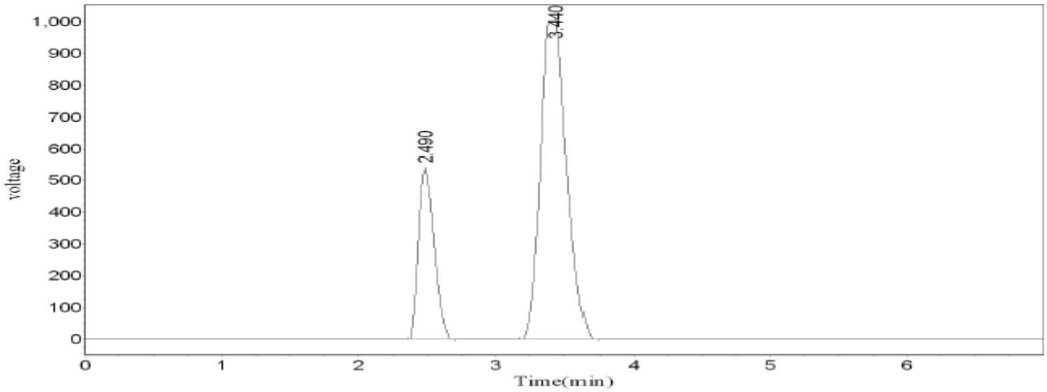
Level	Amount of Famotidine added (µg/ mL)	Amount of ibuprofen added (µg/ mL)	Total volume (mL)
50%	4	120	50
100%	8	240	50
150%	12	360	50



Chromatogram of 50% recovery

Results for chromatogram of 50% recovery

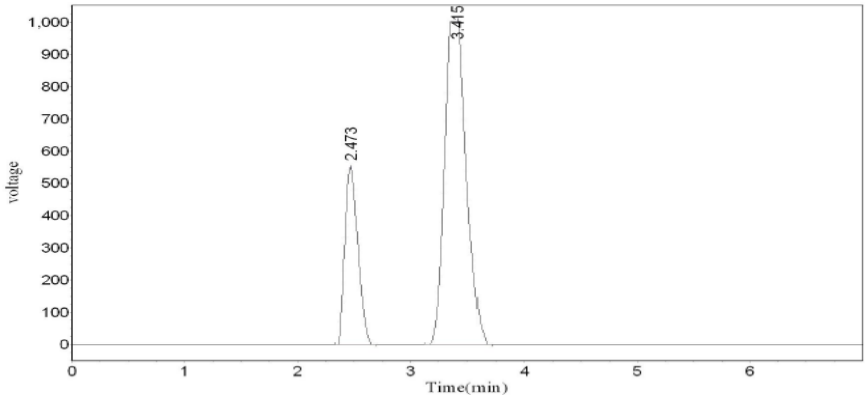
Peak no	Drug	RT	Peak area	TP	Rs	TF
1	Famo	2.482	3844353.750	2783.618	-	1.275
2	Ibu	3.415	11320494	2959.965	3.066	1.156



Chromatogram of 100% recovery

Result for chromatograph of 100% recovery

Peak no	Drug	RT	PA	TP	Rs	TF
1	FAMO	2.498	1722200.625	2755.19	-	1.294
2	IBU	3.432	5996133.5	2990.3	3.077	1.169



Chromatogram of 150%recovery

Results for chromatogram of 150% recovery

Peak no	Drug	RT	PA	TP	Rs	TF
1	FAMO	2.473	4612953	2855.1	-	1.244
2	IBU	3.415	14054761	2690.3	3.097	1.169

The Amount found and Amount added for Famotidine and Ibuprofen and the individual recovery and mean recovery values were calculated and reported in table:

Results for recovery of Famotidine

Concentration	Amount present (µg/ mL)	Amount added (µg/ mL)	Amount found(µg/mL)	% recovery	%mean recovery
50%	7.8	6.5	14.32	100.3	100.7
100%	7.8	7.8	15.86	15.86	
150%	7.8	9.3	17.3	17.3	

Results for recovery of Ibuprofen

Concentration	Amount present (µg/ mL)	Amount added (µg/ mL)	Amount found(µg/mL)	% recovery	% mean recovery
50%	240	192	432	99.9	98.3
100%	240	240	476	98.3	
150%	240	288	531	101.0	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

B) PRECISION:

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

$$\% \text{ RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Procedure for Precision:

Preparation of stock solution:

Accurately weighed and transferred tablet powder equivalent to 240 mg of Famotidine and 8 mg of Ibuprofen working standard into a 100ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (2400, 80µg/ml)

From this, 5 ml of the solution was pipetted into another 50ml volumetric flask and diluted up to the mark with diluent.

Procedure:

The solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the

area of five replicate injections was found to be within the specified limits.

INJECTION	FAMOTIDINE		IBUPROFEN	
	RT	AREA	RT	AREA
1	2.472	1745269.875	3.397	6017053
2	2.548	1744223.250	3.477	6061793.5
3	2.498	1755032.750	3.440	6084698
4	2.473	1745274.75	3.398	6017163.5
5	2.548	1744271.25	3.482	6061885.0
6				
AVERAGE		1746614		6048520
SD		4726.359		30151.48
%RSD		0.270		0.498

Acceptance criteria:

The relative standard deviation of 6 determinations of Retention time and peak areas for Ibuprofen and Famotidine for precision should be less than 2.0%.

Observation:

From the data given in table 8.37, the relative standard deviation (%RSD) of 6 determinations of Retention time and peak areas for Ibuprofen and Famotidine for precision was found to be within the acceptance criteria of less than 2.0%.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more 2%**.**

D) INTERMEDIATE PRECISION/RUGGEDNESS:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution:

Accurately weighed and transferred tablet powder equivalent to 240 mg of Famotidine and 8 mg of Ibuprofen working standard into a 100ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (2400, 80µg/ml)

From this, 5 ml of the solution was pipetted into another 50ml volumetric flask and diluted up to the mark with diluent.

Procedure:

The solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INJECTION	FAMOTIDINE		IBUPROFEN	
	RT	AREA	RT	AREA
1	2.490	1723623.625	3.423	5925015.5
2	2.515	1709983.375	3.448	5864329.5
3	2.492	1720330.625	3.418	5891368
4	2.488	1723516.625	3.423	5924883.5
5	2.498	1720296.625	3.423	5909737.5
AVERAGE		1719549.6		5903066
SD		5590.41		25688.72
%RSD		0.325		0.435

Acceptance criteria:

The relative standard deviation of 5 determinations of Retention time and peak areas for Famotidine and Ibuprofen for precision should be less than 2.0%.

Observation:

From the data given in table 8.37, the relative standard deviation (%RSD) of 6 determinations of Retention time and peak areas for Famotidine and Ibuprofen for precision was found to be within the acceptance criteria of less than 2.0%.

5.4.5 Limit of Detection:

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

$$LOD=3.3(\frac{SD}{S})$$

Observation:

For this method, the LOD value was found to be 0.121 µg/ mL for Famotidine and 3.332 µg/ mL for Ibuprofen.

5.4.6 Limit of Quantification

The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

$$LOQ=10(\frac{SD}{S})$$

Observation: In this method, the LOQ value was found to be 0.404 µg /mL for Famotidine and 11.1 µg/mL for Ibuprofen

5.4.7 Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation were made to evaluate the impact on the method.

Preparation of stock solution:

Standard solution was prepared and analysed using the varied flow rates along with method flow rate.

5.4.7.1 Effect of Variation of Flow Rate:

The method is robust only in flow condition (0.7 mL/min and 0.9 mL/min)

(LESS FLOW).

s.no	Drug	RT	AREA	TP	Rs	TF
1	FAMO	2.798	2064214	2928	-	1.287
2	IBU	3.907	6913134	2801.5	3.24	1.139

s.no	Drug	RT	AREA	TP	Rs	TF
1	FAMO	2.282	1325238.5	2959.6	-	1.255
2	IBU	3.132	4655425.5	2722.3	3.054	1.134

On evaluation of the results, it can be concluded that the variation in flow rate has not affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10 %.

5.4.7.2 Effect of Variation of Mobile Phase Ratio:

The Organic composition in the Mobile phase was varied. Standard was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results were reported in **Table:**

Less organic

s.no	Drug	RT	AREA	TP	Rs	TF
1	FAMO	2.715	1932647.25	2786.37	-	1.295
2	IBU	3.698	6571296.0	2995.9	3.089	1.149
More organic (40:60)						
s.no	**Drug**	**RT**	**AREA**	**TP**	**Rs**	**TF**
1	FAMO	2.515	1709983.3	2786.37	-	1.295
2	IBU	3.448	5864329.5	2995.9	3.089	1.149

On evaluation of the results, it can be concluded that the variation in 10% Organic composition in the mobile phase has not affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase.

Parameter	Value	Famotidine		Ibuprofen	
		RT	area	RT	area
	0.6 mL	2.798	2064214	3.907	6913134
Flow rate	0.8 mL	2.473	1701986	3.407	5899512
	1.0 mL	2.282	1325238.5	3.132	4655425.5
	60:40	2.715	1932647.25	3.698	6571296
Mobile phase	50:50	2.473	1701986	3.407	5899512
(organic phase)	40:60	2.493	1722163.12	3.427	5964

System Suitability Parameters:

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method.

A system suitability evaluation usually contains its own set of parameters.

System suitability parameter Results were reported in **Table:**

Results for system suitability of Famotidine:

Injection	RT	Peak area	TP	Rs	TF
1	2.473	1701986	2783.618	-	1.275
2	2.482	1722200.625	2755.191	-	1.294
3	2.498	1722163.125	2.755.191	-	1.294
4	2.493	1701988.1	3073.310	-	1.275
5	2.473	1701977.35	2675	-	1.279
6	2.483	1722168.34	2783.618	-	1.294
mean		1715450			
SD		11659.89			
%RSD					

Results for system suitability of Ibuprofen

Injection	RT	Peak area	TP	Rs	TF
1	3.407	5987775.750	2859.965	3.066	1.156
2	3.432	5996133.5	2990	3.775	1.169
3	3.407	5899512	2895	3.079	1.169
4	3.427	5995988.5	2619.4	3.758	1.156
5	3.426	5996133.75	2755.19	3.077	1.186
6	3.409	5899512.5	2959.96	3.066	1.179
Mean		5963898			
SD		55707.71			
%RSD		0.934			

Acceptance criteria

The % RSD for the peak area responses Famotidine, Ibuprofen peaks from 6 replicate injections of each standard solution should not be more than 2.0%.

The number of theoretical plates (*N*) for the Famoidine, Ibuprofen peaks should not be less than 2000.

The Tailing factor (TF) for the Famotidine, Ibuprofen peaks should not be more than 2.0.

The resolution (R_s)between the two drugs should be more than 2.0

Observation

The % RSD for the retention times and peak areas of Famotidine, Ibuprofen were found to be less than 2%. The number of theoretical plates of Famotidine, Ibuprofen is found to be within the limit i.e. more than 2000. The tailing factor for Famotidine, Ibuprofen are found to be within the limits. The tailing. The resolution for Famotidine, Ibuprofen is found to be satisfactory and within the limits.

8.4.2 Specificity

Preparation of blank solution The Mobile phase, OPA buffer: Acetonitrile (50:50) was taken as blank solution.

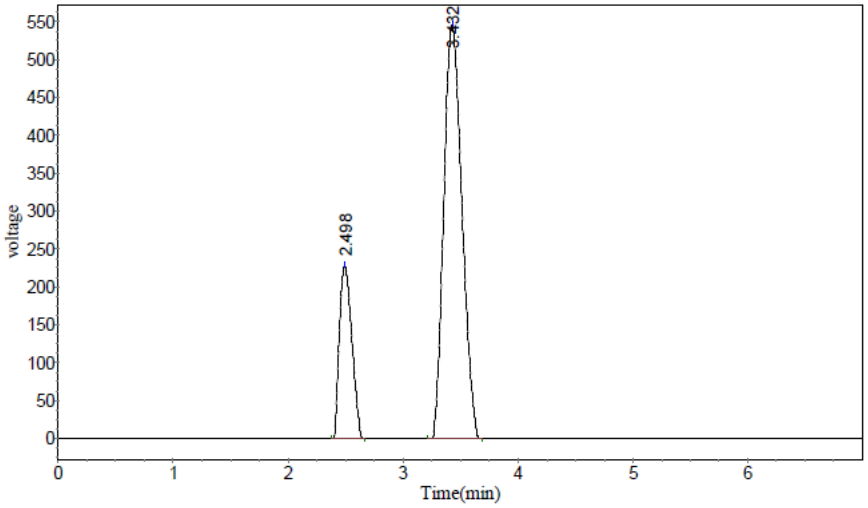
Preparation of standard solution (For preparation refer to chapter 7.2.2.b)

Tablet sample preparation (For preparation refer to chapter 7.2.2.d)

The above prepared solutions were injected and the chromatograms were recorded for the same. The chromatogram for blank is shown in Figure 8.13, the chromatogram for the standard solution is given in Figure 8.14 and the results of the chromatogram are given in Table 8.18, the chromatogram for the test sample i.e tablet sample is given in Figure 8.15 and the results of the chromatogram are given in Table 8.19.

Standard chromatograph

s.no	Peak name	RT	Peak area	TP	Rs	TF
1	FAMO	2.482	1701986	2783	-	1.275
2	IBU	3.407	5899512	2959	3.066	1.156



From the above chromatograms, the resultant data was observed that the diluent or the excipient peaks are not interfering with the ibuprofen and Famotidine peaks

LINEARITY:

Accurately weighed and transferred tablet powder equivalent to 2.6 mg of Famotidine and 80 mg of Ibuprofen working standard into a 100ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (26, 800µg/ml)

Preparation of Level – I (2.6 ppm of Famotidine and 80 of Ibuprofen**):**

1.0ml of the above solution has taken in 10ml of volumetric flask diluted up to the mark with diluent

Preparation of Level – II: (5.2 ppm of Famotidine and 160 of Ibuprofen**)**

2.0ml of the above solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – III (7.8 ppm of Famotidine and 240 of Ibuprofen**):** 3ml of the above solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – IV (10.4 ppm of Famotidine and 320 of Ibuprofen**)**

4.0 ml of the above solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (13.0 ppm of Famotidine and 400 of Ibuprofen**):**

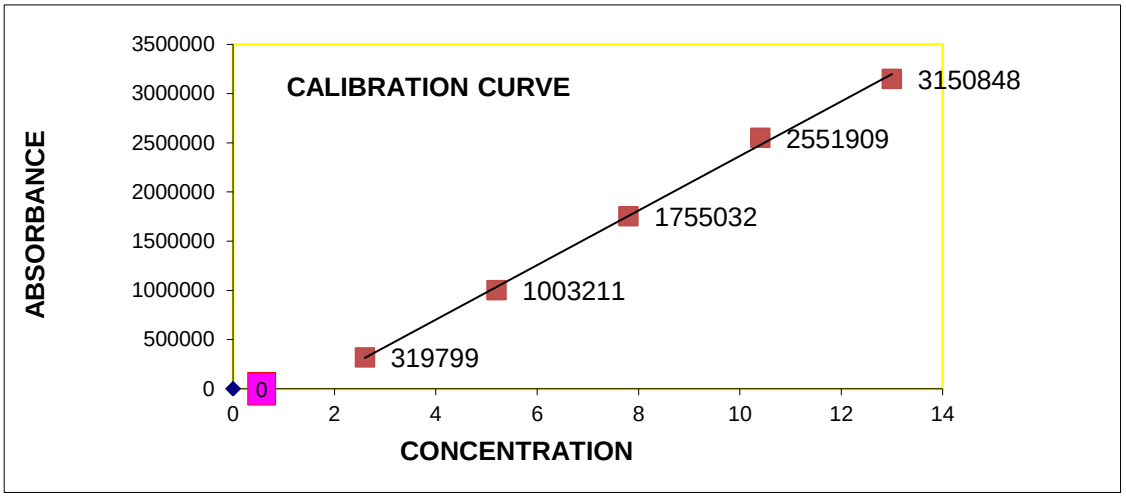
5.0 ml of the above solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Procedure: Each level of solution was injected into the chromatographic system and the peak areas were measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.

Sample preparation for linearity:

Preparation	Volume from standard stock transferred in mL	Volume madeup in mL(with diluents)	Concentration obtained	
			Famotidne	Ibuprofen
1	1.0	10	319799	1677519
2	2.0	10	1003211	3739380
3	3.0	10	1755032	6084698
4	4.0	10	2551909	8174726
5	5.0	10	3150848	10315646

S.NO	CONCENTRATION	PEAK AREA
1	2.6	319799
2	5.2	1003211
3	7.8	1755032
4	10.4	2551909
5	13	3150848



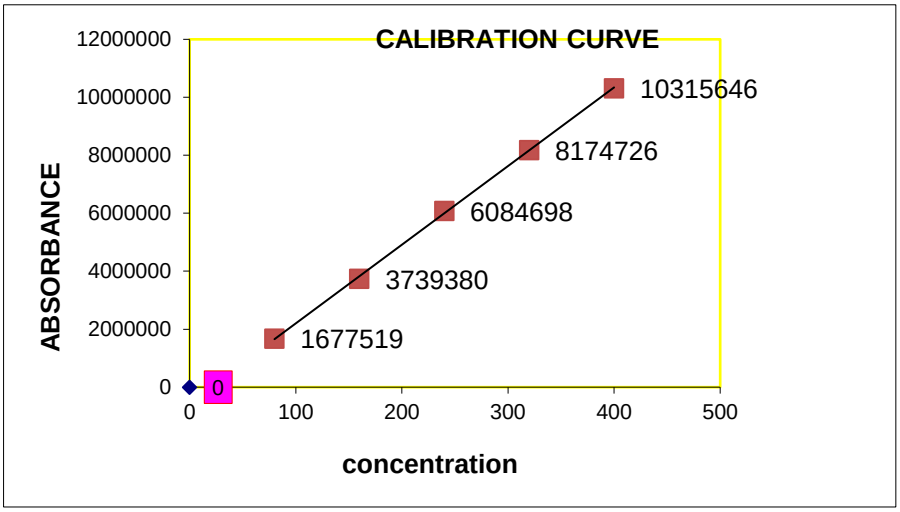
Graph for linearity data of famotidine

$y = 277338.3X - 407079$

$R^2 = 0.9991$

Linearity data for Ibuprofen

S.NO	CONCENTRATION	PEAK AREA
1	80	1677519
2	160	3739380
3	240	6084698
4	320	8174726
5	400	10315646



Graph for linearity data of ibuprofen

y=27139.5X-515086

R^2 = 0.9998

Acceptance Criteria: Correlation coefficient should be not less than 0.999

From the linearity data in Table 8.29**,** the correlation coefficient for linear curve obtained between Concentration vs. Area for standard preparations of Famotidine and ibuprofen are 0.999, 0.9998, respectively. The relationship between the concentration of Famotidine and ibuprofen and area of Famotidine and ibuprofen is linear in the range examined since all points lie in a straight line as shown in Figures 8.21, 8.22 and 8.23 respectively and the correlation coefficient is well within limits.

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