



**RESEARCH ARTICLE**

**Simultaneous HPLC Determination of Ketoprofen and Famotidine in Laboratory Mixture**

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**ABSTRACT**

A simple, specific, precise and accurate reversed phase liquid chromatographic (RP-LC) method has been proposed for the simultaneous determination of ketoprofen and famotidine in laboratory mixtures. The chromatographic separation was performed on a LiChrosorb C<sub>18</sub>, 125 mm x 4.6 mm, 5 µm column at a detector wavelength of 230 nm and a flow rate of 1.5 ml/min. The mobile phase was composed of phosphate buffer (pH adjusted to 7.4 with ortho-phosphoric acid) and acetonitrile (20:80 v/v). The retention times of ketoprofen and famotidine were found to be 3.05 and 6.96 min, respectively. The method was validated for the parameters like specificity, linearity, precision, accuracy, ruggedness, limit of quantitation and limit of detection. The calibration curves were linear in the concentration range of 25.00-200.0 µg/ml for ketoprofen and 5.00-40.00 µg/ml for famotidine. The % recovery for both drugs was in the range between 98.33% and 99.85% with RSD values not greater than 2.55. The presented method for the simultaneous determination of ketoprofen and famotidine in synthetic mixture is specific, rapid and simple with good sensitivity.

**KEYWORDS**

Ketoprofen, Famotidine, HPLC, Validation, Laboratory Mixture, Quality Control

**INTRODUCTION**

Nonsteroidal anti-inflammatory drugs (NSAIDs) are often used for their anti-inflammatory, analgesic, antipyretic effect. Ketoprofen (KET) – RS 2-(3-benzoylphenyl)propionic acid, belongs to group of NSAIDs and possess well established analgesic and antipyretic properties used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and gout<sup>1</sup>. There are some side effects of NSAIDs like ulcers, diarrhoea, dyspepsia, gastric ulceration/bleeding which are life threatening.

Attempts are made to reduce the risk of gastrointestinal toxicity with the application of proton pump inhibitor, for the patients on long term treatment with NSAIDs. Combination of NSAID with a H<sub>2</sub>-receptor antagonists is beneficial as it suppress gastric acid secretion<sup>2</sup>.

Famotidine (FAM) is chemically 3-([2-(diaminomethyleneamino) thiazol-4-yl] methylthio)-N-sulfamoylpropanimidamide. It is commonly used in the treatment of peptic ulcer disease and gastro esophageal reflux disease. Famotidine is histamine H<sub>2</sub>-receptor antagonist which blocks the action of histamine on stomach cells and reduces acid production. Famotidine is useful in promoting the healing of stomach and duodenal ulcers and reducing ulcer pain. Famotidine has been effective in

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preventing recurrence of ulcers when given in low doses for prolonged periods of time<sup>3,4</sup>.

The literature reports many analytical methods for the quantitative determination of ketoprofen<sup>5-11</sup> and famotidine<sup>12-26</sup> alone or in combination with other drugs including spectrophotometry, spectrofluorimetry, liquid chromatography, capillary electrophoresis, polarography, differential pulse voltammetry, potentiometry, Fourier transform infrared spectrophotometry. However, there is no evidence in the literature for simultaneous determination of both drugs.

The aim of the present study was to develop and validate a HPLC method for the simultaneous determination of ketoprofen and famotidine in laboratory mixture contained 100 mg ketoprofen and 20 mg famotidine. The method described complied with validation requirements of ICH<sup>27,28</sup> and could be used for routine quality control of pharmaceutical formulations in ordinary laboratories.

## MATERIALS AND METHOD

### Chemicals and Reagents

Working standards of ketoprofen RS (purity 100.1 %) and famotidine RS (purity 99.87 %) were provided by (Sigma-Aldrich). LC-grade acetonitrile and ortho-phosphoric acid were procured from Merck (Germany). All other chemical reagents were of analytical grade.

### Instrumentation and Chromatographic Conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C<sub>18</sub>, 125 mm x 4.6 mm, 5 µm column eluted with a mixture of phosphate buffer (pH adjusted to 7.4 with ortho-phosphoric acid) and acetonitrile (20:80 v/v) as the mobile phase at flow rate of 1.5 ml/min. Detection was carried out by absorbance at 230

nm. The analysis was carried out at an ambient temperature and injection volume was 20 µl.

### Preparation of Standard Solutions

**For Ketoprofen:** 50 mg of ketoprofen was accurately weighed and transferred to a 50 ml volumetric flask and volume was made up to 50 ml with methanol (Stock solution A-1000 µg/ml).

**For Famotidine:** 20 mg of famotidine was accurately weighed and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol (Stock solution B-200 µg/ml).

**For Mixed Standard:** From the stock solutions A and B dilutions of different concentration were made as mentioned in the Table 1.

Table 1: Preparation of mixed standards

Stock solution → Volume taken (ml)		Total volume (ml)	Concentration in µg/ml	
A	B		KET	FAM
5	5	200	25	5
5	5	100	50	10
5	5	50	100	20
15	15	100	150	30
10	10	50	200	40

### Preparation of Synthetic Mixture

A bulk mixture of both drugs was prepared using 100 mg of KET and 20 mg of FAM. Common excipients which are used in tablet formulation were added in this laboratory mixture, triturated well and weighed. A powder equivalent to 50 mg of KET and 10 mg of FAM was weighed accurately and transferred to 100 ml of volumetric flask, dissolved in sufficient quantity of methanol and volume was adjusted up to the mark with methanol. The stock

solution was filtered through a 0.45  $\mu\text{m}$  Nylon syringe filter and 5.0 ml of the filtrate was diluted into a 25.0 ml volumetric flask to give a test solution containing 100  $\mu\text{g/ml}$  KET and 20.00  $\mu\text{g/ml}$  FAM.

## RESULTS AND DISCUSSION

The proposed method was validated with respect to selectivity, linearity, precision, accuracy, ruggedness, limit of quantitation (LOQ) and limit of detection (LOD) according to ICH requirements to show it could be used for simultaneous determination of ketoprofen and famotidine in laboratory mixture.

### Selectivity

From the chromatogram shown in Fig. 1, it is evident, that under the chosen chromatographic conditions ketoprofen ( $T_r=3.05$  min) and famotidine ( $T_r=6.96$  min) were completely separated. The specificity of the proposed method was confirmed by injecting blank sample. The specificity analysis revealed the HPLC method did not suffer interference by the formulation excipients, since there were not another peaks on the retention times of ketoprofen and famotidine.

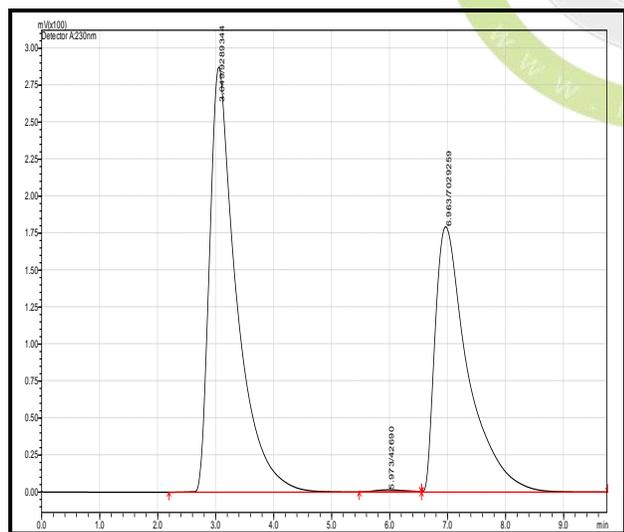


Figure 1: Chromatogram from Analysis of Sample

### Linearity

Calibration curves were constructed in the range of 25.00-200.0  $\mu\text{g/ml}$  for KET and 5.00-40.0  $\mu\text{g/ml}$  for FAM to encompass the expected

concentration in measured samples. An excellent correlation existed between the peak areas and the concentrations of both compounds as can be seen from correlation coefficients. The limit of quantitation and limit of detection were calculated from the standard deviations and slopes of the responses using a signal-to-noise ratio as per ICH guidelines. Data concerning linearity and sensitivity of the method were shown in Table 2.

Table 2: Linearity Data

Parameter	Ketoprofen	Famotidine
Linearity range	25.00-200.0 $\mu\text{g/ml}$	5.00-40.0 $\mu\text{g/ml}$
Slope	12584	15412
Intercept	-1261	1587
Regression coefficient	0.9998	0.9999
Limit of quantitation, ng	10	16
Limit of detection, ng	4	8

The LOQs for ketoprofen and famotidine were found to be 0.5  $\mu\text{g/ml}$  and 0.8  $\mu\text{g/ml}$ , while the LODs were 0.2  $\mu\text{g/ml}$  and 0.4  $\mu\text{g/ml}$ , respectively.

### Accuracy

The accuracy of the method was determined by calculating the recoveries of KET and FAM by the standard addition method. Known amounts of standard solutions of both KET and FAM (50, 100, and 150%) were added to prequantified sample solutions of drug formulation. The method was found to be accurate with recoveries of 98.33%–99.85% and an acceptable RSD of not more than 3% at each level. The recoveries obtained by the proposed method for KET and FAM were shown in Table 3.

Table 3: Results from Study of Accuracy

Amount of sample (µg/ml)		Sets	Amount drug of spiked (µg/ml)		Average amount recovered (µg/ml)		Mean recovery (%) ± SD		% RSD	
KET	FAM		KET	FAM	KET	FAM	KET	FAM	KET	FAM
50	10	1	0	0						
50	10	2	0	0	49.74	9.88	99.49±1.15	98.77±1.80	1.15	1.82
50	10	3	0	0						
50	10	1	25	5						
50	10	2	25	5	74.81	14.93	99.74±0.57	99.55±2.54	0.57	2.55
50	10	3	25	5						
50	10	1	50	10						
50	10	2	50	10	99.41	19.66	99.41±0.90	98.33±2.14	0.87	2.18
50	10	3	50	10						
50	10	1	75	15						
50	10	2	75	15	124.8	24.91	99.85±0.68	99.64±1.40	0.68	1.40
50	10	3	75	15						

Table 4: Precision of the Method

Drug	Interday precision		Intraday precision	
	% Amount found± SD*	% RSD	% Amount found± SD*	% RSD
KET	99.52±0.56	0.56	100.4±0.47	0.47
FAM	98.93±0.69	0.70	99.21±0.39	0.39

\*Average of six determinations

Table 5: Ruggedness of the Method

Analyst	Drug	Label claimed, mg	% Amount found± SD*
Analyst I	KET	100	99.21±0.97
	FAM	20	19.08±1.25
Analyst II	KET	100	100.87±0.68
	FAM	20	19.25±0.94

\*Average of three determinations

## Precision

Precision is determined by studying the repeatability and intermediate precision. Repeatability (interday precision) result indicates the precision under the same operating conditions over a short interval of time. Intermediate precision study expresses within laboratory variation in different days. In both inter- and intraday precision study the values of % RSD were not more than 2.0% indicates good repeatability and intermediate precision (Table 4).

## Ruggedness Study

It expresses the precision within laboratories, variation like different analyst. Ruggedness of the methods was assessed by carrying out assay 3 times with different analyst by using same equipment. In Table 5 results from ruggedness investigations were presented.

## CONCLUSION

The newly developed RP-LC method for simultaneous determination of ketoprofen and famotidine in laboratory mixture is specific, precise, accurate and rapid. Hence the proposed method is suitable for the quality control of the raw materials, formulations and dissolution studies.

## ACKNOWLEDGMENT

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