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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF IBUPROFEN AND FAMOTIDINE BY RP HP-LC

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V. Krishna Vaishnavi[1], Prof Dr. M. Madhuri[2],
Department of Pharmaceutical Analysis, CES College of Pharmacy, Kurnool

ABSTRACT:

A validated, simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the concurrent estimation of Ibuprofen and Famotidine. The separation was achieved using an XBridge Amide column (250×4.6 mm, 5 µm particle size).

The optimized mobile phase consisted of an ammonium formate buffer mixed with acetonitrile in a ratio of 15:85 (v/v), with the pH adjusted to 3 using formic acid. A flow rate of 0.8 mL/min was maintained, the column temperature was held at 25°C, and analytes were detected at a wavelength of 265 nm.

The method exhibited excellent linearity over a concentration range of 25% to 150% for both drugs, with a high correlation coefficient (R^2) of 0.998 for both Ibuprofen and Famotidine. The precision was confirmed by low relative standard deviation values of 0.7% for Ibuprofen and 0.5% for Famotidine. The calculated limits of detection (LOD) and quantification (LOQ) were 0.3221 µg/mL and 0.976 µg/mL for Ibuprofen, and 4.62 µg/mL and 14.066 µg/mL for Famotidine, respectively

Keywords: *RP-HPLC, Ibuprofen and Famotidine, Ammonium Formate Buffer, Accurate*

INTRODUCTION:

Introduction to Pharmaceutical Analysis

Pharmaceutical analysis is a major discipline within pharmaceutical sciences that focuses on the identification, separation, and quantitative evaluation of chemical substances present in raw materials, drug products, and biological samples. It provides the scientific framework necessary to ensure the safety, efficacy, identity, purity, and quality of pharmaceutical compounds throughout their lifecycle.

A variety of analytical techniques such as chromatography, spectroscopy, and titrimetric are applied to determine the concentration of drugs, detect impurities and degradation products, study dissolution behavior, and evaluate stability under various conditions.

The field is broadly divided into:

Qualitative Analysis: Methods used to establish the presence or absence of specific chemical constituents in a sample.

Quantitative Analysis: Procedures used to determine the exact concentration or percentage of one or more analytes.

Pharmaceutical analysts play an essential role in guaranteeing that drug formulations meet

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regulatory standards and therapeutic expectations. With the advent of sophisticated instrumentation, the field has transformed into a highly precise and sensitive domain capable of detecting even trace quantities of substances.

Overview of Analytical Techniques

Spectrometric Techniques

Spectrometric methods rely on the interaction of electromagnetic radiation with matter. These techniques are used extensively for qualitative and quantitative evaluation of metals, organic compounds, and biomolecules.

S. No	Technique	Principle	Applications
1.	Plasma Emission Spectroscopy	Excitation in high-temperature plasma produces atomic emission	Trace-level determination of metals and non-metals
2.	Flame Emission Spectroscopy	Flame excitation causes atomic emission	Analysis of alkali and alkaline earth metals
3.	Atomic Absorption Spectroscopy	Atomized elements absorb specific wavelengths	Detection of trace metals and some non-metals
4.	Atomic Fluorescence Spectroscopy	Excited atoms emit fluorescence	Analysis of mercury and hydrides of non-metals
5.	X-ray Emission Spectroscopy	Electron bombardment induces emission	Geological and metallurgical sample analysis
6.	UV–Visible Spectroscopy	Electronic transitions in molecules absorb UV/visible light	Measurement of unsaturated organic compounds
7.	Infrared Spectroscopy	Molecular vibrations absorb IR radiation	Identification of organic structures
8.	NMR Spectroscopy	Nuclear spin transitions in a magnetic field	Structural elucidation of organic compounds

Chromatographic Techniques

Chromatography separates analytes based on their interaction between a stationary and a mobile phase.

S. No	Technique	Principle	Applications
1.	Thin Layer Chromatography	Differential migration across stationary phase	Qualitative separation of mixtures
2.	Gas Chromatography	Partition between gas mobile and liquid stationary phase	Analysis of volatile compounds
3.	HPLC	Interaction between analyte, mobile phase, and stationary phase	Quantification of non-volatile compounds
4.	Ion-Exchange Chromatography	Reversible exchange of ions	Analysis of ionic species
5.	Size-Exclusion Chromatography	Separation based on molecular size	Characterization of polymers and proteins
6.	Affinity Chromatography	Specific binding to complementary ligands	Purification of biomolecules
7.	Vapour Phase Chromatography	Migration through liquid stationary phase with gaseous mobile phase	Determination of volatile analytes
8.	Chiral Chromatography	Interaction with chiral stationary phase	Enantiomeric separation
9.	Supercritical Fluid Chromatography	Use of supercritical fluids as mobile phase	Environmental, biomedical, and food sample analysis

High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is an advanced chromatographic technique widely used for the separation and

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quantification of pharmaceuticals. The system uses high pressure to push the mobile phase through columns packed with particles of small diameter, resulting in enhanced resolution, faster analysis, and improved efficiency.

Modes of HPLC

a) Normal-Phase Chromatography (NP-HPLC)

Stationary phase: polar

Mobile phase: non-polar

Best suited for non-polar compounds

b) Reverse-Phase Chromatography (RP-HPLC)

Stationary phase: non-polar

Mobile phase: polar

Suitable for polar and moderately polar molecules

Comparison of NP-HPLC and RP-HPLC

Parameter	NP-HPLC	RP-HPLC
Stationary Phase Polarity	High	Low
Mobile Phase Polarity	Low	High
Elution Order	Non-polar → polar	Polar → non-polar
Effect of Increasing Mobile Phase Polarity	Decreases retention	Increases retention

Instrumentation of HPLC

HPLC instrumentation generally includes:

- Solvent Reservoirs
- Pumps
- Sample Injector
- Column Assembly
- Detector
- Data Acquisition System / Integrator

Solvent Reservoirs

These containers hold the mobile phase. Prior to entering the pump, solvents undergo:

Mixing – either in low-pressure or high-pressure mixing chambers

Degassing – to remove dissolved gases using vacuum, helium purging, or ultrasonication

Pumps

Pumps deliver the mobile phase at high pressure (1000–3000 psi) to overcome resistance from tightly packed columns. Common pump types include:

- Syringe pumps
- Reciprocating pumps (single or dual piston)
- Diaphragm and pneumatic pumps

Sample Injectors

Manual or automated injectors introduce a fixed volume of sample into the flowing mobile phase.

Columns

HPLC columns consist of:

- Guard column: protects the analytical column
- Analytical column: performs actual separation

Column properties include:

- Material: stainless steel
- Length: 5–30 cm
- Diameter: 2–50 mm
- Particle size: 1–20 µm
- Stationary phases: C18, C8, C4, phenyl, cyano, amino, silica

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Detectors

Detectors monitor eluting analytes. Common detectors include:

Type	Responds To	Examples
Bulk-property detectors	Changes in mobile phase	Refractive index, conductivity
Solute-property detectors	Analyte properties	UV, fluorescence, electrochemical detectors

Method Development in HPLC

Steps in Method Development

- Define method requirements and separation goals
- Collect information on analyte and sample
- Select initial mode, detector, column, and mobile phase
- Generate preliminary chromatograms
- Fine-tune critical parameters
- Optimize and finalize the method

Defining Separation Goals

Method goals may include specifications for resolution, sensitivity, linearity, and analysis time. Typical acceptance criteria:

- Resolution > 2
- %RSD < 2% for precision
- Linear range covering 80–120% of target concentration
- Short, cost-effective analysis time

Gathering Sample Information

- Number of analytes
- Chemical structure
- Functional groups
- pKa, solubility, λmax
- Availability of reference standards
- Safety and stability data

Initial Method Development

a. Selection of Chromatographic Mode: Reverse-phase chromatography is the most commonly used for small organic molecules.
b. Detector Selection: UV/Vis detectors are preferred when analytes have UV absorbance. Non-chromophoric compounds may require RI or ELSD detectors.
c. Column Selection: Silica-based C18 or C8 columns (3–5 µm particles) are typical starting points.
d. Mobile Phase Selection: Includes choice of buffer (phosphate, acetate, formate), pH adjustment based on analyte pKa, and organic solvents such as acetonitrile or methanol.
e. Trial Chromatograms: Initial chromatograms help identify retention behavior and required adjustments.
f. Fine Tuning: Parameters optimized include organic solvent %, pH, flow rate, gradient, column type, and detector wavelength.
g. Optimization: A method is considered optimized when it consistently meets all performance criteria.

Analytical Method Validation (ICH Guidelines)

Validation ensures that an analytical method consistently produces reliable and accurate results.

Importance of Validation

- Ensures product quality
- Minimizes errors and batch failures
- Supports regulatory compliance
- Enhances process control

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- Facilitates scale-up and routine quality testing

Types of Validation

- Process validation
- Analytical method validation
- Cleaning validation
- Revalidation

Analytical Method Validation Parameters

a. Linearity: Ability to obtain results proportional to concentration over a defined range.
b. Range: Interval between upper and lower analyte levels where the method demonstrates acceptable precision, accuracy, and linearity.
c. Precision: Expressed as %RSD; includes repeatability and intermediate precision.
d. Accuracy: Closeness between the measured value and true value, expressed as percent recovery.
e. Specificity: Ability to measure analyte response in the presence of impurities, degradants, or excipients.
f. Detection Limit (LOD): Lowest analyte amount detectable but not quantifiable.
g. Quantitation Limit (LOQ): Lowest analyte amount quantifiable with suitable precision and accuracy.
h. Robustness: Ability of a method to remain unaffected by small deliberate variations in analytical parameters.

System Suitability Testing

System suitability tests confirm that the analytical system is performing properly before sample analysis.

Parameter	Acceptance Criteria
Theoretical Plates (N)	> 2000
Capacity Factor (k')	> 2
Resolution (Rs)	> 2
Tailing Factor (Tf)	< 2
%RSD	< 2%

CHEMICAL PROFILE:

Drug Profile and Combination Therapy: Ibuprofen and Famotidine

This content provides a summary of the pharmacological properties of Ibuprofen and Famotidine, along with details on their fixed-dose combination product, Duexis, suitable for an article publication.

Pharmacological and Physical Characteristics

Ibuprofen is a propionic acid derivative and a prototypical non-steroidal anti-inflammatory drug (NSAID). It presents as a white to off-white crystalline powder with a melting point range of 74–78°C. It is practically insoluble in water but highly soluble in organic solvents such as ethanol, methanol, acetone, and chloroform. Ibuprofen has a pKa value of approximately 4.91 and is classified under the Biopharmaceutics Classification System (BCS) as Class II, indicating low solubility and high permeability. The typical adult dosage ranges from 800 mg per day up to a maximum daily dose of 3200 mg.

Famotidine is a white to pale yellow crystalline compound classified as a histamine H2-receptor

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antagonist, which works by reducing gastric acid secretion. It has a melting point between 161–163°C. Solubility characteristics show it is very slightly soluble in water, slightly soluble in methanol, and freely soluble in glacial acetic acid. Its pKa value is approximately 7.1, and it is classified as BCS Class III, characterized by high solubility and low permeability. For acute therapy of active duodenal ulcers, the typical adult oral dosage is 40 mg once daily at bedtime, with a maximum daily dose for gastroesophageal reflux disease being 40 mg.

The Combination Product: Duexis

Duexis is a fixed-dose combination tablet containing 800 mg of Ibuprofen and 26.6 mg of Famotidine. This product was developed by Horizon Pharma and received FDA approval on April 23, 2011. The tablet is typically administered orally three times a day.

Mechanism of Action: The Ibuprofen component provides analgesic and anti-inflammatory properties by non-selectively inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, thereby blocking prostaglandin synthesis. Famotidine acts as a competitive H2-receptor antagonist to inhibit gastric acid secretion, which helps mitigate the risk of developing upper gastrointestinal ulcers associated with NSAID use.

Metabolism: Both active ingredients are primarily metabolized in the liver, with the elimination half-life for ibuprofen being 2–4 hours and famotidine being 2.5–3.5 hours.

Inactive Ingredients: Each tablet contains standard excipients such as Microcrystalline Cellulose, Croscarmellose sodium, Colloidal anhydrous, Magnesium Stearate, Povidone, and others.

Pharmacopoeial Status

The status of the individual components and select dosage forms in various pharmacopoeias is summarized below:

- **Ibuprofen:** Official in the United States Pharmacopeia (USP), European Pharmacopoeia (EP), British Pharmacopoeia (BP), and Indian Pharmacopoeia (IP).
- **Ibuprofen Tablets:** Official in the USP, BP, and IP.
- **Ibuprofen Gel/Cream/Oral Suspension/ Prolonged Release Tablets:** These forms are primarily official in the BP.
- **Famotidine:** Official in the USP, EP, BP, and IP.
- **Famotidine Tablets:** Official in the USP.

AIM AND OBJECTIVES

The primary aim of this research endeavor was to develop and rigorously validate a novel, simple, precise, accurate, and reliable Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method specifically for the simultaneous quantitative determination of

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Ibuprofen and Famotidine in pharmaceutical formulations.

Objectives: In the analysis of multi-component pharmaceutical formulations, a common challenge arises when one drug interferes with the accurate estimation of another. Traditional methods often necessitate time-consuming and complex sample preparation steps, such as component separation via extraction, which can sometimes compromise overall accuracy and efficiency.

To circumvent these analytical challenges, this study aimed to establish a synchronized research approach capable of estimating both drugs concurrently without mutual interference.

The core objectives were thus:

- To develop an efficient analytical method that allows for the simultaneous quantification of both Ibuprofen and Famotidine within bulk drug materials and commercial tablet dosage forms.
- To ensure the developed method adheres to stringent validation criteria for precision, accuracy, and reliability.

Experimental Plan of Work

The systematic execution of this research followed a structured plan:

1. Literature Review and Drug Characterization: Compiling the essential physical and chemical properties of both Ibuprofen and Famotidine.
2. Chromatographic Optimization:
    - Determining the optimal maximum absorption wavelength (λmax) using a Photodiode Array (PDA) detector.
    - Selecting appropriate chromatographic conditions, including the stationary phase (column selection), mobile phase composition, and flow rate.
    - Systematically optimizing these parameters to achieve optimal separation and resolution.
3. Method Validation: Validating the finalized method according to established regulatory guidelines to confirm its suitability for intended analytical use.
4. Documentation and Reporting: Summarizing the complete methodology, results, and conclusions for final documentation and article publication.

MATERIAL AND METHODS:

Chemicals and Materials

High-purity Active Pharmaceutical Ingredients (APIs) of Ibuprofen (from Granules India Limited) and Famotidine (from SMS Pharmaceuticals Limited) were used. HPLC-grade solvents, specifically Acetonitrile (Merck) and water (Milli-Q-Water system), were utilized. Other reagents included extra-pure Ammonium Formate and Formic Acid (Rankem).

Placebo components used for sample preparation included Microcrystalline Cellulose (Mingtai), Colloidal Silicon dioxide (Evonik),

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Croscarmellose Sodium (Mingtai), and Magnesium Stearate (Peter Graven).

Equipment and Instrumentation

The study utilized a Waters HPLC system equipped with an autosampler and a PDA detector. A Mettler Toledo analytical balance and pH meter were used for precise measurements. Additional laboratory equipment included an Ultra-Sonicator (Life Care Sciences), a centrifuge (DLAB), a vacuum filtration apparatus, and Class A grade volumetric glassware from manufacturers such as Borosil and Tarsons.

Chromatographic Method Development and Optimization

The method was developed using an RP-HPLC technique. A detection wavelength of 265 nm was selected, as this wavelength provided optimal absorbance for both Ibuprofen and Famotidine based on UV spectral analysis.

Chromatographic Conditions:

The optimized stationary phase was an XBridge Amide column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase composition was finalized after several trials exploring different buffers (e.g., Ortho Phosphoric acid, Glycine, Ammonium Formate) and acetonitrile ratios. The optimal mobile phase comprised an Ammonium Formate buffer (adjusted to pH 3 with Formic Acid) and Acetonitrile in a ratio of 15:85 (v/v). A consistent flow rate of 0.8 mL/min was maintained throughout the analysis, and the mobile phase itself served as the diluent for all sample preparations.

Preparation of Key Solutions:

- **Ammonium Formate Buffer (pH 3):** 2.52 g of ammonium formate was dissolved in 1000 mL of HPLC grade water, the pH was adjusted to 3 using formic acid, filtered through a 0.45 µm PVDF membrane, and degassed.
- **Mobile Phase:** A mixture of the buffer and acetonitrile (15:85 v/v) was prepared, degassed, and filtered.
- **Standard Stock Solution (Target Concentrations):** A combined standard solution targeting approximately 640 ppm (µg/mL) of Ibuprofen and 21.3 ppm (µg/mL) of Famotidine was prepared in the diluent for system suitability and analysis.

Method Validation Procedures

The optimized method was subjected to comprehensive validation in accordance with established guidelines to confirm its reliability.

System Suitability: Prior to analysis, the system suitability was verified using five replicate injections of the standard solution. Acceptance criteria required the relative standard deviation (%RSD) of peak areas and retention times to be NMT (Not More Than) 2.0%. Theoretical plates (N) for both peaks had to be NLT (Not Less Than) 2000, and the tailing factor (T) NMT 2.0.

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Specificity: Specificity testing confirmed the absence of interference from the blank solvent or common excipients (placebo components) at the specific retention times of Ibuprofen and Famotidine, ensuring accurate quantification within complex matrices.

Linearity and Range: The linearity of the method was evaluated across a range of 25% to 150% of the target concentration level. Standard solutions were prepared at six concentration points, injected into the HPLC system, and peak areas were measured. A calibration curve was plotted relating concentration (x-axis) to peak area (y-axis). The acceptance criterion for linearity required a correlation coefficient (R2) of NLT 0.99%.

Precision: Method precision was assessed by analyzing six replicate preparations of a single homogeneous sample solution containing both analytes and placebo components. The %RSD of the peak areas across these six injections was calculated. The acceptance criterion for precision stipulated a %RSD of NMT 2.0%.

Accuracy (Recovery Study): Accuracy was determined by the standard addition method, preparing samples at three concentration levels: 50%, 100%, and 150% of the target concentration. The percentage recovery of the spiked analytes was determined to evaluate the closeness of the measured values to the true values.

Robustness

Robustness analysis was performed to evaluate the reliability of the optimized method when critical operational parameters were intentionally varied within minor limits. This study ensures that minor environmental or procedural deviations during routine laboratory analysis do not significantly affect the assay results. The chosen variations were applied to the flow rate and the column temperature.

Assay Procedure

The final optimized and validated method was applied for the quantitative determination (Assay) of Ibuprofen and Famotidine in prepared samples containing both active pharmaceutical ingredients (APIs) and common excipients (placebo).

Standard Solution Preparation: A standard stock solution was prepared containing target concentrations of 640 ppm of Ibuprofen and 21.3 ppm of Famotidine by dissolving accurately weighed API samples in the mobile phase diluent.

Sample Solution Preparation: A representative sample mixture containing Ibuprofen, Famotidine, and placebo excipients was accurately weighed, dissolved in diluent with sonication, centrifuged to remove solid excipients, and filtered to achieve the final test concentration.

Chromatographic Analysis: Ten microliters of the blank solution, standard solution, and sample solution were injected into the HPLC system. Six replicate injections of the sample solution were

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made to determine the final assay percentage and calculate the precision of the measurement.

RESULTS:

Method development trials using various chromatographic conditions failed to achieve adequate separation and optimal peak characteristics for both Ibuprofen and Famotidine. Early trials with an X-terra RP18 column and buffers at pH 7.11 and pH 2.5 resulted in either no elution, only one peak, or low retention times and poor efficiency. A trial with a Zorbax SCX column showed improved resolution and efficiency, but the first peak eluted too quickly. More information is available in the provided text.

DISCUSSION

Method Optimization: The analytical method was optimized by systematically evaluating various chromatographic parameters. A detection wavelength of 265 nm was chosen due to optimal absorbance characteristics for both compounds. The optimal separation was achieved using an XBridge Amide column and an isocratic mobile phase composed of an ammonium formate buffer (pH 3) and acetonitrile in a ratio of 15:85 (v/v), delivered at a flow rate of 0.8 mL/min. This mobile phase composition yielded symmetrical peaks with satisfactory retention times (Ibuprofen at ~3.2 min and Famotidine at ~8.6 min). he total run time was fixed at an efficient 12 minutes.

Method Validation: The developed method was fully validated according to the International Council for Harmonisation (ICH) Q2(R1) guidelines.

Linearity and Range: The method demonstrated excellent linearity across the range of 25% to 150% of the target concentration for both drugs. The correlation coefficients (R2 values) were close to unity (0.9996 for Ibuprofen and 0.9997 for Famotidine), confirming a strong linear relationship between concentration and peak area within the tested range.

Precision: High precision was observed, with low relative standard deviation (%RSD) values (0.7% for Ibuprofen and 0.5% for Famotidine) obtained from replicate analyses, indicating excellent method repeatability.

Accuracy: Accuracy was confirmed through recovery studies at 50%, 100%, and 150% concentration levels. Mean recovery percentages were consistently within acceptable limits (100–101% for Ibuprofen and 98–100.6% for Famotidine), demonstrating the method's ability to quantify the analytes accurately in the presence of excipients.

Robustness: The method proved robust to minor variations in flow rate and column temperature, with all critical parameters remaining within established acceptance criteria.

Sensitivity: The method showed adequate sensitivity, with calculated Limits of Detection (LOD) and Quantification (LOQ) of 0.3221 µg/mL and 0.976 µg/mL for Ibuprofen, and 4.62

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µg/mL and 14.066 µg/mL for Famotidine, respectively

CONCLUSION

A novel, simple, accurate, precise, and reliable Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) analytical method has been successfully developed and validated for the simultaneous quantification of Ibuprofen and Famotidine in pharmaceutical dosage forms.

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