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"Advanced Analytical Approach for Ketotifen Fumarate Quantification from marketed dosage form: A Precision RP-HPLC Validation Study"

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Abstract:
Ketotifen fumarate is a popular prescription medication, particularly in pediatric formulations. The focus of this study was driven by the importance of ensuring drug quality, as substandard medications can negatively impact human health and overall quality of life. Consequently, it became crucial to develop and validate an analytical method to accurately quantify the active drug in marketed formulations.

In this study, an optimized and validated reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed for the quantification of ketotifen fumarate in marketed dosage forms. The optimized HPLC conditions utilized a mobile phase composed of methanol and 0.04M Na_2 HPO_4 buffer (50:50) with a flow rate of 1 mL/min. The method demonstrated excellent selectivity and sensitivity.

The calibration curve for ketotifen fumarate was established with the formula y = 27714x - 7912 (R^2 = 0.996), covering a concentration range of 10–60 µg/mL. The method's accuracy and intermediate precision were evaluated using both standard solutions and samples spiked with known amounts of ketotifen fumarate. The recovery values ranged between 80-110%, with a relative standard deviation (RSD) of less than 7.4% for both intraday and interday analyses.

The ketotifen content in the sample (n=6) was determined to be 16.574 ± 0.098 µg/mL. In conclusion, the RP-HPLC method was successfully validated and applied for the accurate determination of ketotifen fumarate in marketed dosage forms, demonstrating its reliability for routine quality control.

Keywords: Ketotifen fumarate, UV, RP-HPLC, Method Development, and Validations etc.

I. Introduction:

Ketotifen fumarate is an antihistamine and mast cell stabilizer widely used in the management of allergic conditions and asthma. Developed initially as a non-competitive H1 receptor antagonist, it has gained popularity for its dual mechanism of action, making it particularly effective in treating chronic allergic disorders such as allergic rhinitis, conjunctivitis, urticaria, and bronchial asthma. Its ability to both preventsand treat allergic symptoms has made it a key drug in pediatric formulations, where allergies are a common concern.

The pharmacological action of ketotifen fumarate involves two main pathways: histamine receptor antagonism and mast cell stabilization. By blocking histamine H1 receptors, ketotifen prevents histamine, a compound released during allergic reactions, from binding to its receptors and causing typical allergic symptoms like itching, swelling, and redness. In addition to this, ketotifen also stabilizes mast cells, preventing them from releasing other inflammatory mediators such as leukotrienes, which are responsible for the exacerbation of allergic responses. This dual action makes ketotifen fumarate highly effective in both the prevention and treatment of allergic reactions.

In cases of asthma, ketotifen is used more as a preventive agent. While it may not be effective in treating an acute asthma attack, it can significantly reduce the frequency of asthma attacks when used regularly. By inhibiting the release of inflammatory mediators, ketotifen helps prevent airway inflammation and bronchoconstriction, thus improving respiratory function over time.

Clinical Uses: Ketotifen fumarate is commonly prescribed for a range of allergic conditions:

1.Allergic Rhinitis: Also known as hay fever, this condition is characterized by sneezing, a runny or stuffy nose, and itchy eyes. Ketotifen is used to alleviate these symptoms.

2.Allergic Conjunctivitis: In the form of eye drops, ketotifen helps manage itchy, red, and watery eyes caused by exposure to allergens.

3. Chronic Urticaria: For individuals suffering from hives or welts on the skin, ketotifen helps reduce itching and swelling.
4. Asthma Prophylaxis: Though not a first-line treatment for asthma, ketotifen is used as a prophylactic to reduce the frequency and severity of asthma attacks.
5. Atopic Dermatitis: For patients with eczema or atopic dermatitis, ketotifen can help manage the inflammatory response and reduce flare-ups.

Safety and Side Effects:

Ketotifen fumarate is generally well-tolerated, with mild and transient side effects. Common side effects include drowsiness, dry mouth, dizziness, and weight gain, particularly with oral formulations. In rare cases, ketotifen may cause more serious side effects such as liver dysfunction or hypersensitivity reactions. Its sedative properties, although mild, should be taken into account when prescribing to children or patients who may need to operate machinery.

Importance in Pediatric Use:

Ketotifen fumarate is particularly significant in pediatric medicine due to its safety profile and efficacy in managing allergies and asthma. Pediatric populations are often more prone to allergic reactions, and the availability of ketotifen in syrup and eye drop formulations provides a practical and effective solution for young patients. The use of ketotifen in asthma prophylaxis is especially valuable, as it reduces the need for more potent and potentially harmful medications like corticosteroids.

Figure 01: Marketed dosage form of Ketotifen fumarate.

II. Experimental:

Materials and Chemicals: The reference standards of ketotifen fumarate were obtained from a local distributor in Dhule, Maharashtra, India. Methanol, of liquid chromatography grade, was used as the solvent in this research. Additionally, sodium hydrogen phosphate, required for the buffer solution, was sourced from Merck Millipore.

Chromatographic Conditions:

An Agilent 1100 series HPLC system equipped with a Luna Phenomenex® C18 column (250 × 4.6 mm, 5 µm, end-capped) was utilized in this study. Additional equipment included an OHAUS® PA 413 analytical balance, a Gast® vacuum pump, a Retsch® ultrasonicator, and a set of Socorex® micropipettes. The data obtained from the HPLC analysis were processed using Excel with Agilent Lab Advisor software to generate a linear calibration model and perform other data calculations.

HPLC Conditions

An isocratic RP-HPLC method was employed using a mobile phase composed of methanol and 0.04 M Na2HPO4 buffer at pH 4.8 in a 50:50 ratio, with a flow rate set to 1 mL/min. The column temperature was maintained at 26.5°C. The wavelength for detection was optimized at 287 nm, and a sample injection volume of 10 µL was used for each analysis.

Preparation of standard solution

Accurate weights of 10.6mg for ketotifen fumarate and cyproheptadine hydrochloride, respectively, were put into different 10 mL volumetric flask. The concentration of ketotifen fumarate was 1000 ppm.

Preparation of sample solution

An accurate weight of 100 mg of divided powder sample were placed in beaker glass and diluted by methanol into the volume of 10.0 mL volumetric flask. The working sample solution was prepared by transferring a 1.5 mL sample diluted solution into a 5 mL volumetric flask followed by dilution with methanol to volume. This solution was filtered using a sterile PTFE membrane before injection into the HPLC system.

Preparation of spiked sample solution

An accurate weighed of 100 mg ketotifen fumarate were placed in beaker glass and diluted by methanol into the volume of 10.0 mL volumetric flask, respectively. The spikedsample solution was prepared by transferring 110, 130,150 µL ketotifen fumarate solution into a 5 mL volumetric flask followed by dilution with methanol to volume. This solution was filtered using a sterile PTFE membrane before injection into the HPLC system.

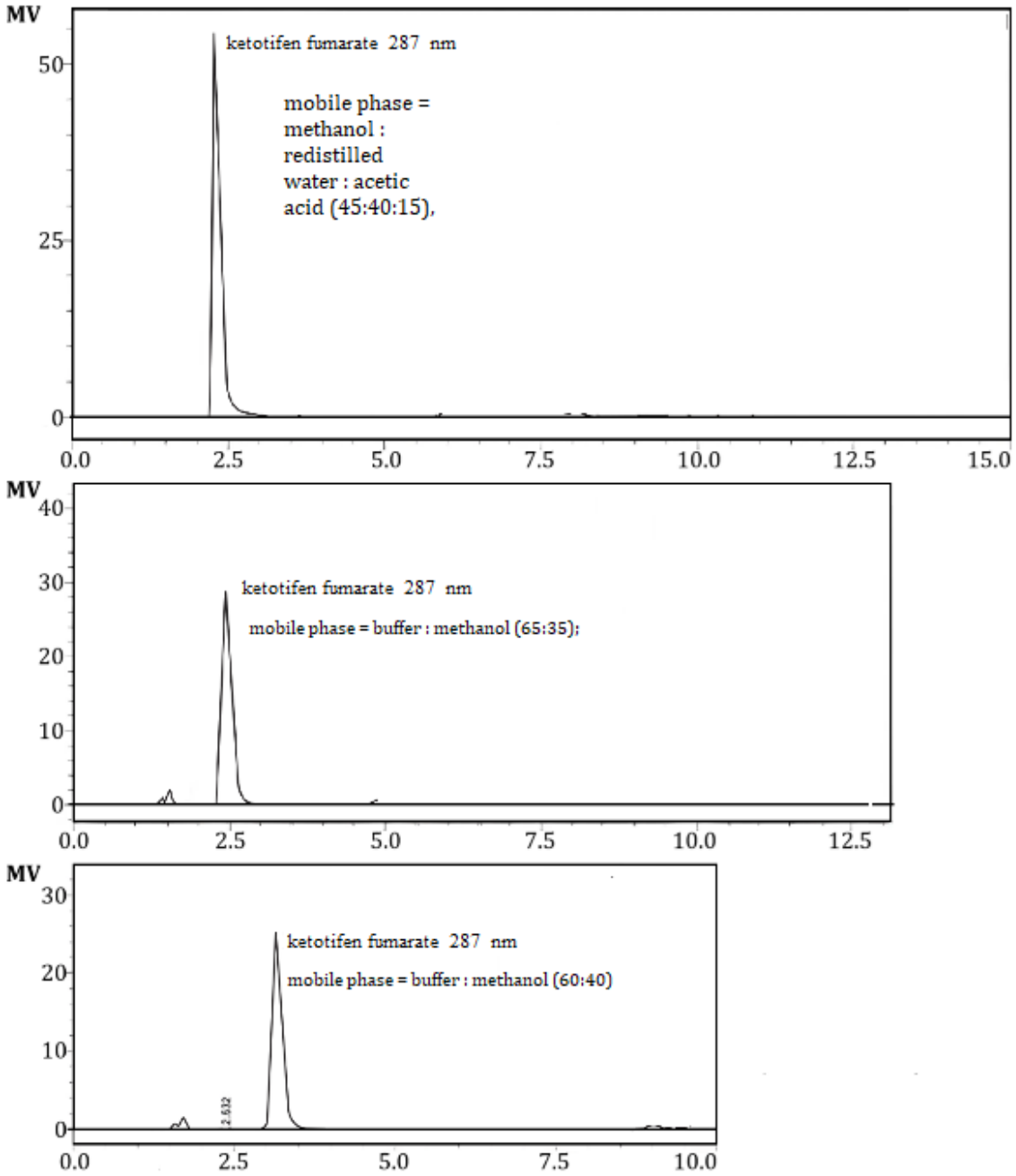

Figure 1: Optimization of Analytical Method ketotifen fumarate marketed formulation.

Optimization of Analytical Method

Optimization of the chromatographic separation was performed by variation of mobile phase. Wavelength detection was set to 287 nm in which two analytes were intersecting wavelengths. The volume injection for each run was 10 µL. The composition of each run was methanol: redistilled water: acetic acid (45:40:15), buffer: methanol (35:65), buffer: methanol (40:60) with the same flow rate 0.8 mL/min (Figure 1).

Validation of Analytical Method

The HPLC method was validated for selectivity, linearity, range, precision, accuracy, and sensitivity, including limits of detection (LOD) and quantitation (LOQ), in compliance with the AOAC Official Methods of Analysis. This ensures reliable and reproducible results for the intended analytical application.

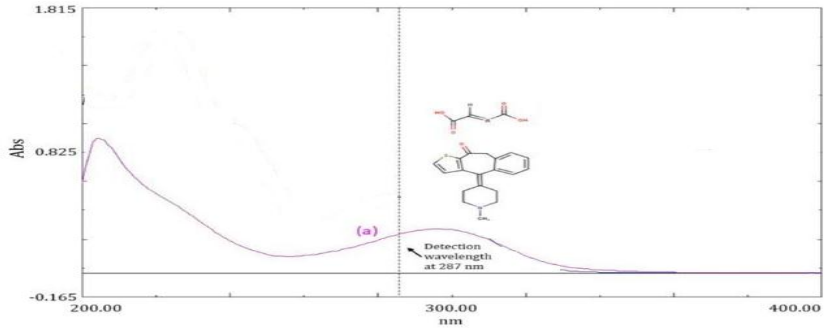

Figure 2: Uv-Visible spectra of ketotifen fumarate marketed formulation.

III. Results and Discussion:

Determination of Wavelength Detection:

Wavelength detection should be stated at the initial stage of RP-HPLC method development. It was important to choose an optimal wavelength for detecting several compounds in a mixture matrix (Figure 2). In this study, detection of the analytes was performed at 287 nm. At this wavelength, ketotifen fumarate can be optimally detected. The extensive overlapping spectra of analytes may become the limitation while applying the UV spectroscopy. However, by applying RP-HPLC method, these analytes can be detected at the same wavelength and resulted separation profile for each analyte due to the interaction of the analyte with mobile and stationary phase at the various interaction strength.

Table 1: Results of system suitability test (n=6):

Analytes	Retention Time		Area	
	Mean	**RSD (%**	**Mean**	**RSD (%)**
Ketotifen Fumarate	3.472	1.561	271556.8	0.973

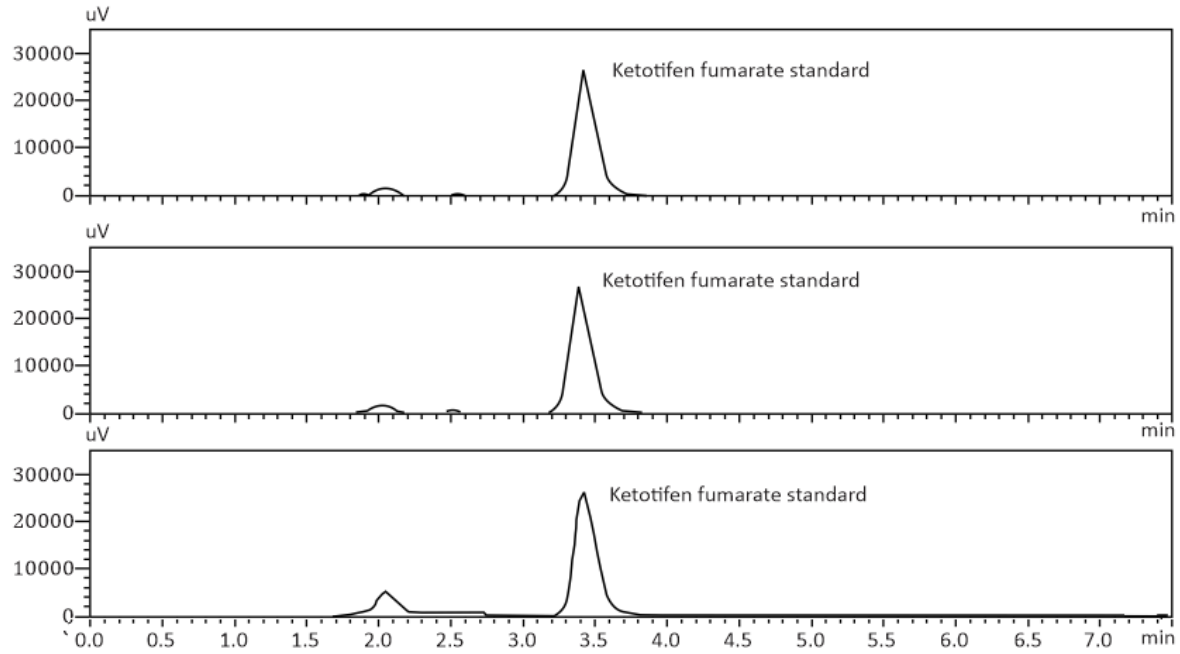

Figure 3: RP-HPLC separation profiles ketotifen fumarate Mobile phase: methanol: buffer (50:50 v/v). Column: C18 Luna Phenomenex® (250×4.6 mm, i.d. 5 µm) encapped. Flow rate: 1.0 mL/min. Column temperature: 26.5 °C. Wavelength detection at 287 nm. Volume injection: 10 µL

System Suitability Test: The system suitability test ensures the performance of systems and analytical methods. Key RP-HPLC parameters include retention time and peak area, with the relative standard deviation (RSD) for both meeting

the acceptance criteria (RSD ≤ 2%) as shown in Table I.

Selectivity: Selectivity measures the ability to quantify the analyte without interference, typically through a peak purity test. The RP-HPLC method showed a single peak with no interference. It met the criteria of resolution > 1.5, tailing factor < 2.0, and theoretical plate number > 2000, with minimal deviation (≤ 2%) for both retention time and peak area for all analyzed compounds. The retention time of ketotifen fumarate was 3.44 minutes (Figure 3).

Linearity and Range: Peak areas versus concentrations of standard solutions were plotted to create calibration curves for ketotifen fumarate quantification. The calibration curve equation for ketotifen fumarate was y = 27714x - 79111 (R^2 = 0.9945), showing linearity in the range of 10.9–54.5 µg/mL.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Sensitivity parameters LOD and LOQ were determined using the standard deviation approach. LOD for ketotifen fumarate was 2.889µg/mL, and LOQ was 9.6312 µg/mL.

Accuracy and Precision of Standard Solutions**: The accuracy and intermediate precision were evaluated at three concentration levels (low, medium, high), analyzed in triplicate on different days (interday) and on the same day (intraday). The RP-HPLC method met the acceptance criteria with RSD < 7.3% and recovery between 80-110% (Table II), confirming its accuracy and precision for analyzing ketotifen fumarate standard solutions.

Table II.

Table 2: Accuracy and Precision Study of Standard Ketotifen Fumarate (n=3):

Intraday						
Compounds	Level	Added amount (µg/mL)	Found amount (µg/mL)	SD	RSD (%)	Recovery (%)
Ketotifen Fumarate	Low	10.7	12.8	0.01	0.18	86.34
	Medium	21.8	21.2	0.06	0.31	102.67
	High	32.7	32.7	0.55	1.69	99.89
Interday						
Ketotifen Fumarate	Low	10.8	12.5	0.09	0.61	85.64
	Medium	21.8	21.1	0.06	0.31	102.67
	High	32.6	32.1	0.55	1.68	99.87

Table 3: Accuracy and Precision Study of Spiked Samples (n=3):

Intraday						
Compounds	Level	Added amount (µg/mL)	Found amount (µg/mL)	SD	RSD (%)	Recovery (%)
Ketotifen Fumarate	Low	15	15.52	0.13	0.86	103.48
	Medium	13	11.35	0.18	1.59	87.34
	High	11	11.19	0.15	1.37	101.73
Interday						
Ketotifen Fumarate	Low	15	15.58	0.15	0.99	103.88
	Medium	13	11.36	0.17	1.50	87.44
	High	11	10.49	0.40	3.90	95.41

Table 3. Results of Determination of Ketotifen Fumarate:

Analyte concentration (µg/mL)	
Replication	**Ketotifen Fumarate**
1	16.56
2	16.56
3	16.60
4	16.64
5	16.36
6	16.63
7	16.63

Mean (µg/mL)	16.57
SD	0.09
RSD (%)	0.59

Accuracy and Precision of Spiked Samples: Accuracy and intermediate precision of spiked samples were tested by adding standard ketotifen fumarate solutions to blank samples at three concentration levels, analyzed in triplicate over three days (interday). Results in Table III indicated RSD < 7.3% and recovery within 80-110%, confirming the method's accuracy and precision for analyzing spiked samples with ketotifen fumarate.

Determination of Ketotifen Fumarate in Marketed Dosage Forms: The validated RP-HPLC method was used to determine the content of ketotifen fumarate in marketed dosage forms. Table IV presents the results, showing method precision with RSD < 7.3% across seven replicates, confirming the method's reliability for determining ketotifen fumarate content.

IV. Conclusion:

A validated isocratic RP-HPLC method was successfully developed. This method was selective, linear, sensitive, accurate, and precise to quantitatively analyze ketotifen fumarate marketed dosage form. This method could be applied for routine analysis quality of drug in India. The good quality of drugs can directly impact to patient's health.

Conflict of interest:

Authors don't have any conflict of interest

Acknowledgement:

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