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Development and Validation of a Reverse Phase High Performance Liquid Chromatographic Method for Simultaneous Estimation of Moxifloxacin and Ketorolac Tromethamine in Combined Dosage Form

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KEYWORDS
Moxifloxacin, Ketorolac, RP-HPLC

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Abstract

A simple, specific, accurate, precise, and economical reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for the simultaneous estimation of Moxifloxacin and Ketorolac in combined pharmaceutical dosage form. Chromatographic separation was achieved using an Inertsil ODS C18 column (150 × 4.6 mm, 5 µm) with a mobile phase consisting of mixed phosphate buffer (pH 6.8) and acetonitrile in the ratio of 70:30 (v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 303 nm. The retention times were found to be 2.299 min and 4.806 min for Moxifloxacin and Ketorolac, respectively. The developed method was validated according to ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, LOD, and LOQ. The % recovery was found between 99–101%, and %RSD was less than 2%. The method was found to be suitable for routine quality control analysis of the combined dosage form.

Introduction

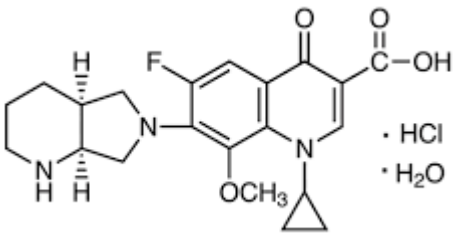
Analytical chemistry is defined as the science and art of determining the composition of materials in terms of elements or compounds present (Ahuja and Scypinski, 2001). Modern pharmaceutical analysis relies heavily on instrumental techniques due to their specificity, sensitivity, and reproducibility. High Performance Liquid Chromatography (HPLC), introduced commercially in 1969, has become one of the most widely used analytical techniques for quantitative pharmaceutical analysis (Dong, 2006; Snyder et al., 1997). HPLC offers

advantages such as speed, precision, automation capability, and high resolution. Moxifloxacin is a fourth-generation fluoroquinolone antibiotic active against Gram-positive and Gram-negative bacteria. It acts by inhibiting DNA gyrase and topoisomerase IV, enzymes essential for bacterial DNA replication. Ketorolac is a non-selective COX inhibitor with potent analgesic and anti-inflammatory properties. It inhibits prostaglandin synthesis by blocking cyclooxygenase enzyme. Several UV and HPLC methods have been reported for individual estimation of Moxifloxacin (Tarkase et al., 2012; Sahu et al., 2011) and Ketorolac (Tsvetkova et al., 2001). However, limited economical RP-HPLC methods are available for their simultaneous estimation in combined dosage forms. Therefore, the present study aimed to develop and validate a sensitive and reproducible RP-HPLC method for simultaneous estimation of both drugs.

Drug Profile

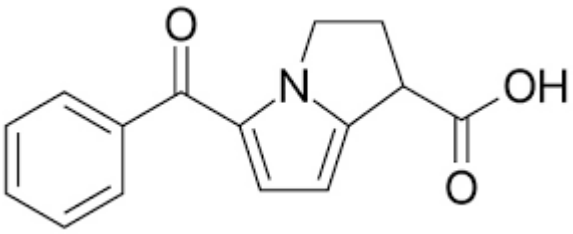
Moxifloxacin

Molecular Formula: C21H24FN3O4
Molecular Weight: 437 g/mol
Category: Broad-spectrum antibacterial
Solubility: Soluble in water and methanol
Mechanism of Action: Inhibits DNA gyrase and topoisomerase IV, preventing bacterial DNA replication
Therapeutic Uses: Acute bacterial sinusitis, chronic bronchitis exacerbations, community-acquired pneumonia



Ketorolac Tromethamine

Molecular Formula: C15H13NO3 (Ketorolac base)
Molecular Weight: 255.27 g/mol (base)
Category: NSAID (Analgesic, Anti-inflammatory)
Mechanism of Action: Inhibits cyclooxygenase (COX-1 and COX-2), reducing prostaglandin synthesis
Therapeutic Uses: Post-operative pain, inflammatory conditions



Materials and Methods

Instruments

- HPLC: Waters 1200 series / Shimadzu LC-2010 CHT
- Column: Inertsil ODS C18 (150 × 4.6 mm, 5 µm)
- UV Spectrophotometer: Shimadzu UV-1800
- pH meter: Thermo Orion

Chemicals

- Moxifloxacin Working Standard
- Ketorolac Working Standard
- Acetonitrile (HPLC grade)
- Methanol (AR grade)
- Potassium dihydrogen phosphate
- Orthophosphoric acid

Method Development

Selection of Wavelength

UV spectra were recorded between 200–400 nm. A common wavelength at 303 nm was selected for simultaneous detection

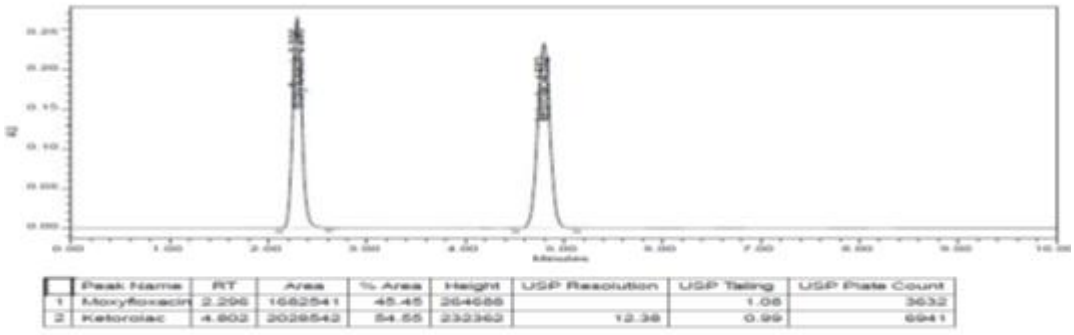
Optimized Chromatographic Conditions

- Column: Inertsil ODS C18
- Mobile phase: Phosphate buffer (pH 6.8) : Acetonitrile (70:30 v/v)
- Flow rate: 1.0 mL/min
- Injection volume: 20 µL
- Detection wavelength: 303 nm
- Run time: 10 min

Validation Parameters (ICH Q2 R1)

- Specificity (acid, base, UV, oxidation stress testing)
- Linearity: 10–60 µg/mL (r^2 = 0.998 and 0.997)
- Accuracy: 80–120% recovery (99–101%)
- Precision: %RSD < 2%
- System precision and method precision evaluated

RESULTS & DISCUSSION



	Peak Name	RT	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Moxyfloxacin	2.296	1662541	45.45	264668		1.08	3632
2	Ketorolac	4.802	2028542	54.55	232362	12.38	0.99	6941

Fig. No.1: Chromatogram for standard solution

Linearity

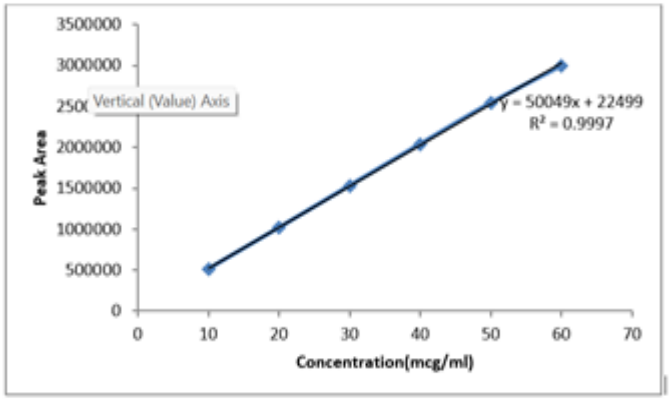


Fig. 2 Linearity plot of Moxyfloxacin

Table No.1: linearity of Moxyfloxacin

Concentration µg/ml	Peak Area
10	431198
20	849053
30	1277780
40	1700843
50	2122524
60	2496793

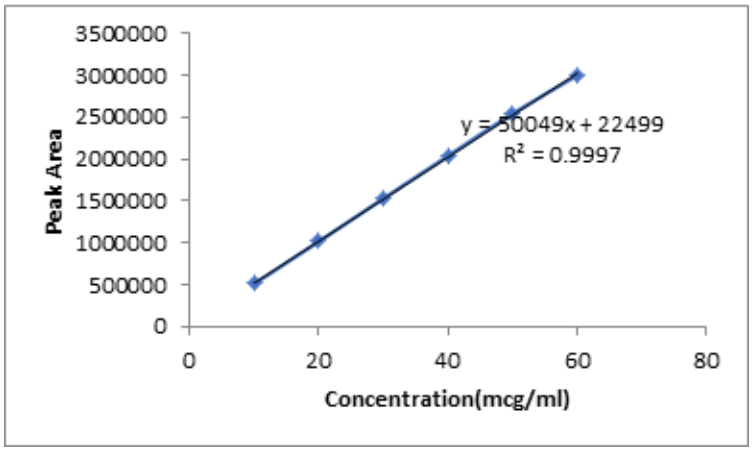


Fig. 3: Linearity plot of Ketorolac

Table No. 2 linearity of Ketorolac

Concentration µg/ml	Peak Area
10	513317
20	1017768
30	1531353
40	2039458
50	2543993
60	2999390

ACCURACY

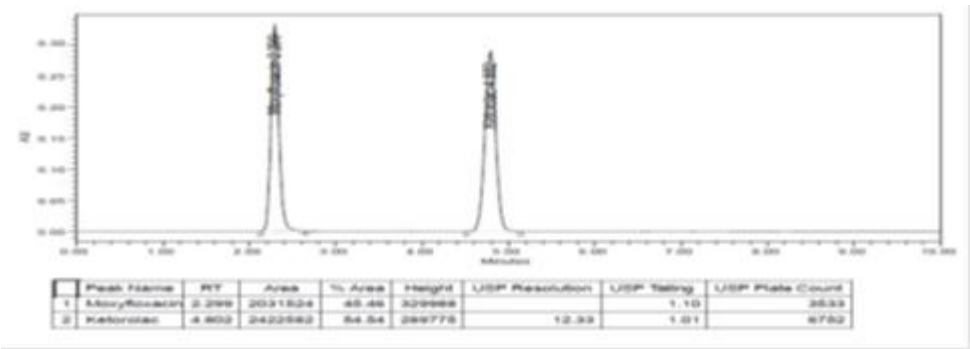
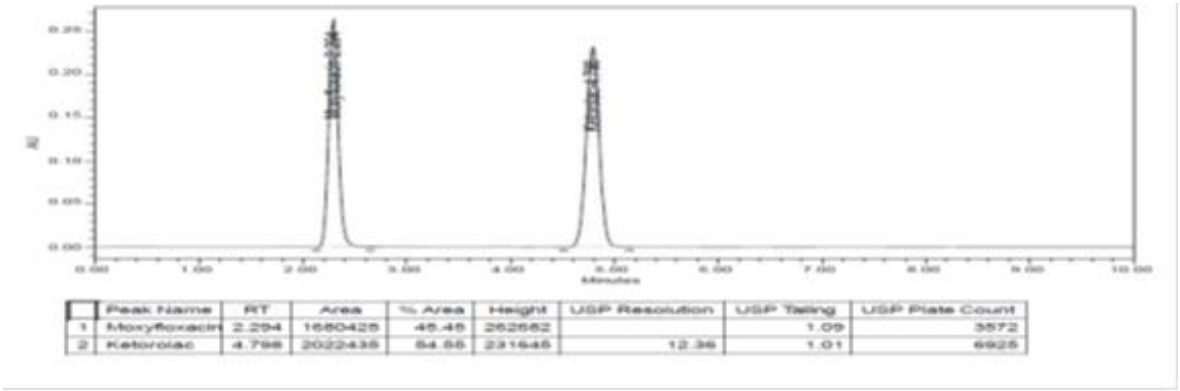


Fig.No.4: Chromatogram for Accuracy 120% at 100%Injection-3

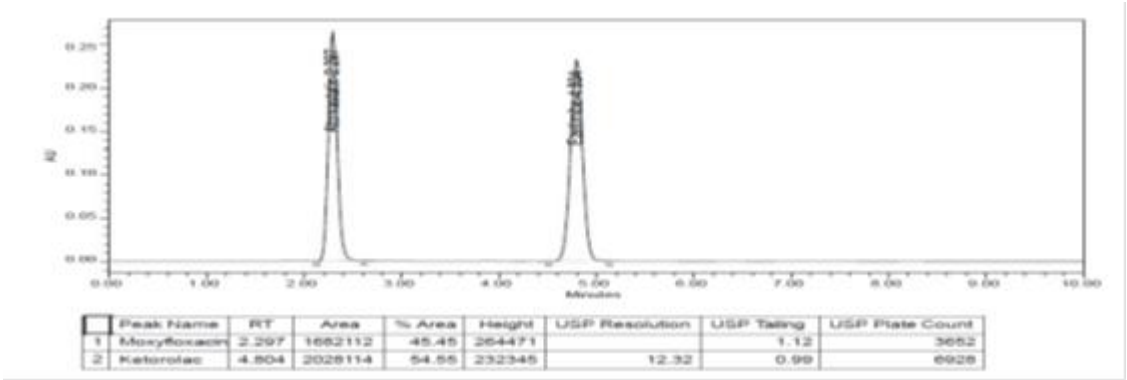
PRECISION



	Peak Name	RT	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Moxyfloxacin	2.294	1680425	45.45	262682		1.09	3572
2	Ketorolac	4.798	2022436	54.55	231646	12.36	1.01	6925

Fig. No. 5: Shows method precision chromatogram Injection-6

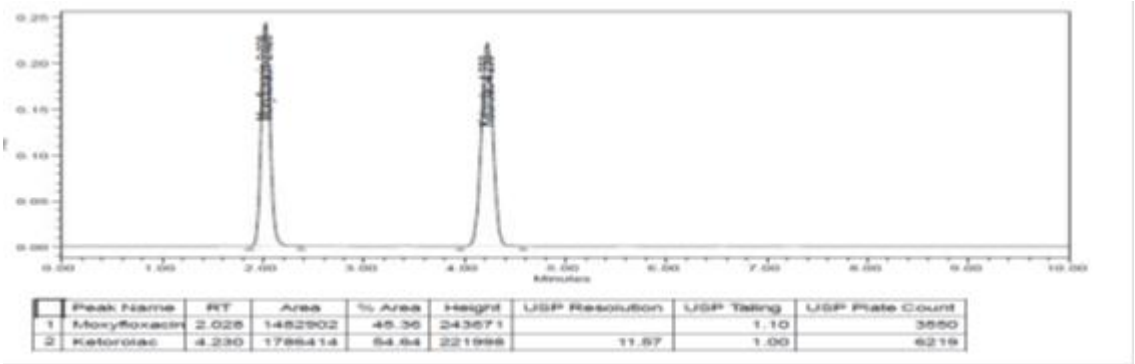
RUGGEDNESS



	Peak Name	RT	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Moxyfloxacin	2.297	1682112	45.45	264471		1.12	3652
2	Ketorolac	4.804	2028114	54.55	232345	12.32	0.99	6928

Fig. No.6: Shows chromatogram of Analyst-2

ROBUSTNESS



	Peak Name	RT	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Moxyfloxacin	2.028	1482902	45.36	243671		1.10	3550
2	Ketorolac	4.230	1786414	54.64	221998	11.57	1.00	6219

Fig. No.7: Shows chromatogram of Flow Rate-2

DISCUSSION

The optimized mobile phase composition (buffer pH 6.8: ACN 70:30) provided sharp, symmetrical peaks with acceptable theoretical plates and resolution. Retention times were 2.299 min and 4.806 min for Moxifloxacin and

Ketorolac respectively. Linearity studies demonstrated excellent correlation coefficients. Recovery studies confirmed absence of interference from excipients. **Stress** studies confirmed specificity under acid, base, UV, and oxidative conditions. The method proved to be precise, accurate, and robust. Compared to previously reported methods, the developed method offers shorter run time, better peak symmetry, and cost-effective mobile phase composition.

CONCLUSION

A simple, rapid, precise, accurate, and economical RP-HPLC method was successfully developed and validated for simultaneous estimation of Moxifloxacin and Ketorolac in combined dosage form. The developed method complies with ICH Q2(R1) guidelines and is suitable for routine quality control analysis in pharmaceutical industries.

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