Innovative RP-HPLC Method Development and Validation for Dual Estimation of Meloxicam and Rizatriptan with Stability Studies

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ABSTRACT

A novel, simple, and stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of Meloxicam and Rizatriptan in bulk and pharmaceutical dosage form. The chromatographic separation was performed on an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of phosphate buffer (pH 3.0) and acetonitrile (40:60 v/v) at a flow rate of 1 mL/min, with UV detection at 215 nm. The retention times for Meloxicam and Rizatriptan were found to be 3.15 min and 2.31 min, respectively. The method exhibited excellent linearity in the concentration ranges of 5–30 µg/mL for Meloxicam and 2.5–15 µg/mL for Rizatriptan, with correlation coefficients of 0.999 for both. The percentage recoveries were within 98–102%, and %RSD values were below 2%, indicating high accuracy and precision. LOD and LOQ were found to be 0.08 µg/mL and 0.24 µg/mL for Meloxicam, and 0.06 µg/mL and 0.17 µg/mL for Rizatriptan, respectively. The proposed method was successfully validated as per ICH Q2 (R1) guidelines and is suitable for routine quality control analysis of these drugs in combined dosage forms.

Keywords: RP-HPLC; Meloxicam; Rizatriptan; Method validation; ICH guidelines; Simultaneous estimation

INTRODUCTION

Meloxicam and Rizatriptan are two pharmacologically distinct drugs often used to manage pain-related disorders. Meloxicam belongs to the class of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and is mainly prescribed for the treatment of osteoarthritis and rheumatoid arthritis due to its potent anti-inflammatory and analgesic effects. The IUPAC name of Meloxicam is 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide. Molecular formula is $C_{14}H_{13}N_3O_4S_2$ and the Molecular weight is 351.401g/mol. The structure of Meloxicam is in Fig 1.

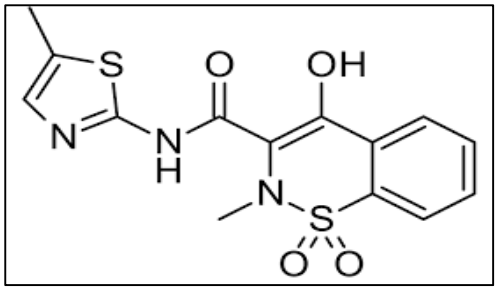

Figure 1: Structure of Meloxicam

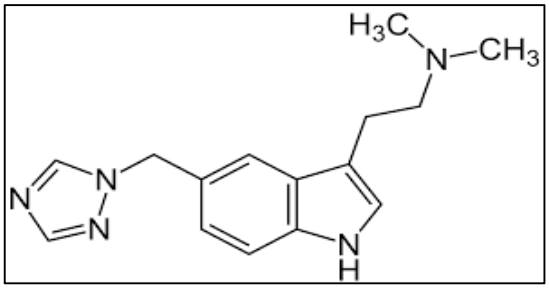

Figure 2: Structure of Rizatriptan

Rizatriptan, on the other hand, is a selective 5-hydroxytryptamine (5-HT$_1$B/1D) receptor agonist used for the acute management of migraine attacks with or without aura. It acts by stimulating serotonin receptors located on intracranial blood vessels and sensory nerves, causing vasoconstriction and inhibition of neuropeptide release, which relieves migraine pain. The structure of Rizatriptan is in Fig 2. The IUPAC name is N,N-dimethyl-2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethanamine. Molecular formula is $C_{15}H_{19}N_5$ and the Molecular weight is 269.3449g/mol.

Literature Survey reveals that there is no RP-HPLC method was developed for the simultaneous estimation of Meloxicam and Rizatriptan. High-Performance Liquid Chromatography (HPLC) remains one of the most reliable analytical techniques for quantifying drugs in pharmaceutical formulations due to its accuracy, sensitivity, and reproducibility. The objective of the present work was to develop a simple, precise, and time-efficient HPLC method for the simultaneous estimation of Meloxicam and Rizatriptan in bulk and combined dosage form, with shorter retention time and well-resolved peaks, followed by validation of the proposed method in accordance with ICH guidelines and stability studies.

EXPERIMENTAL

Materials and Methods:

The APIs of Meloxicam and Rizatriptan were obtained as gift samples from Hetero Drugs Pvt Ltd. Symbravo tablets having the label claim Meloxicam 20mg, Rizatriptan 10mg were taken from the local market. HPLC grade solvents like Acetonitrile, Water and Methanol and AR grade chemicals like Potassium Dihydrogen phosphate and Orthophosphoric acid were obtained from Rankem.

Instruments:

Analysis was carried out by using Microprocessor UV Visible Double Beam, Waters Alliance 2695 HPLC embedded with empower 2 software and TUV detector. The other instruments used were Analytical weighing Balance, Ultrasonicator, pH Meter, Hot air oven, Vortex meter.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions: Accurately weighed 10 mg of Meloxicam and 5mg of Rizatriptan and transferred to 50ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution 1and 2. (200 µg/ml of Meloxicam and 100 µg/ml of Rizatriptan)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20 µg/ml of Meloxicam and 10µg/ml of Rizatriptan)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (200 µg/ml of Meloxicam and 100 µg/ml of Rizatriptan)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20 µg/ml of Meloxicam and 10 µg/ml of Rizatriptan)

RESULTS AND DISCUSSION:

Several trials has been taken for the proper optimization of RP HPLC method by changing different ratio of buffer and acetonitrile and columns.

Chromatographic conditions for Optimized method:

Mobile phase	**:**	KH_2PO_4 : Acetonitrile 40:60v/v
Flow rate	**:**	1 ml/min

Column	**:**	Inertsil ODS C18 (4.6 x 250mm, 5µm)
Detector wave length	**:**	215nm
Column temperature	**:**	25°C
Injection volume	**:**	10µL
Run time	**:**	6 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50

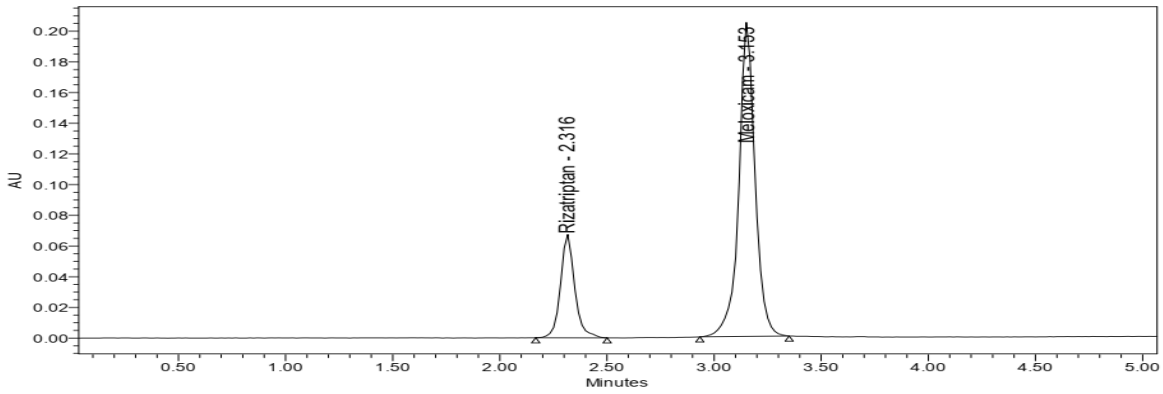

Fig 3: Optimized Chromatogram for Meloxicam and Rizatriptan

System suitability: The system suitability parameters were determined by injecting standard Solutions of 20µg/ml of Meloxicam and 10µg/ml of Rizatriptan six times and the parameters like peak tailing, resolution and USP plate count were determined.

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

Table 1: System Suitability results for Meloxicam and Rizatriptan

	Rizatriptan				**Meloxicam**			
s.no	**RT**	**area**	**Plate Count**	**Tf**	**RT**	**area**	**Plate Count**	**Tf**
1	2.315	105846	5786	1.19	3.153	297326	8817	1.13
2	2.315	105134	5922	1.17	3.153	296524	9055	1.14
3	2.315	105547	6104	1.14	3.154	296332	9064	1.13
4	2.317	106108	6318	1.18	3.154	297316	8934	1.12
5	2.317	105570	6346	1.13	3.156	296865	9025	1.13
6	2.318	105605	6308	1.18	3.156	296437	8999	1.13
Average		***105635***				***296800***		
Stdev		***326.2***				***441.4***		
%RSD		***0.3***				***0.1***		

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

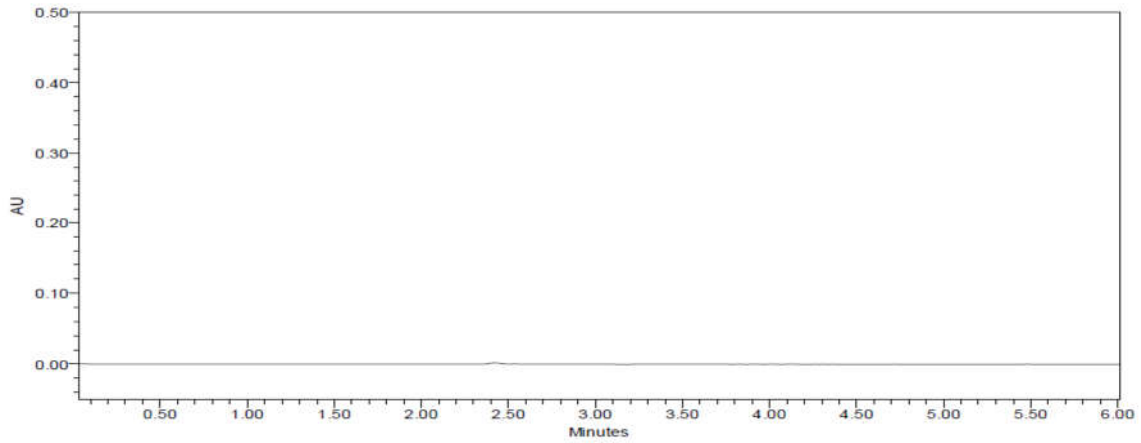

Fig 4: Blank Chromatogram

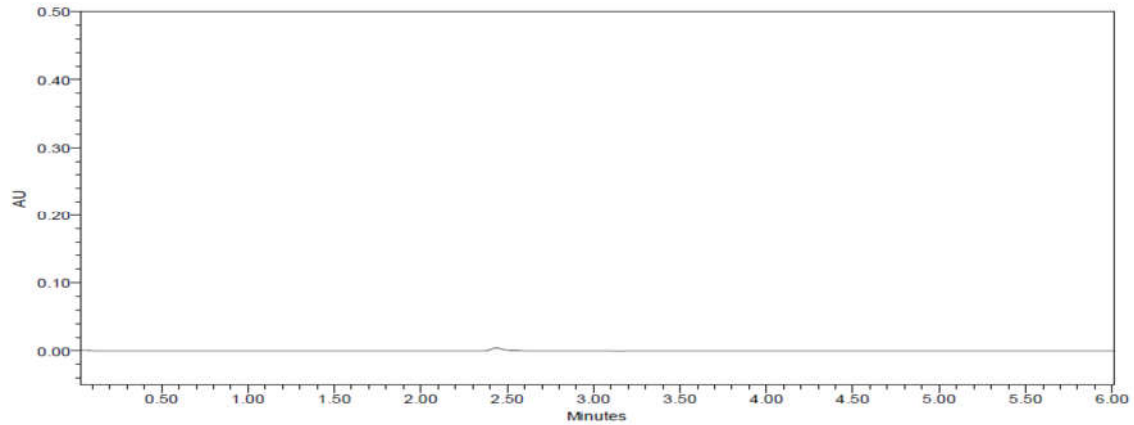

Fig 5: Placebo Chromatogram

Linearity:

Linearity was studied by injecting series of standard solutions of Meloxicam and Rizatriptan in the concentration range of 5-30µg/ml and 2.5-15 µg/ml for Meloxicam and Rizatriptan respectively. Calibration curves were plotted for both the standard drug solutions by taking concentration on X-axis and peak areas on Y-axis. The obtained data were subjected to regression analysis using least squares method.

Table 2 Linearity table for Meloxicam and Rizatriptan

Meloxicam		Rizatriptan	
Conc (µg/mL)	**area**	**Conc (µg/mL)**	**area**
5	74326	2.5	25353
10	148453	5	51615
15	223105	7.5	78790
20	295987	10	105546
25	371995	12.5	129043
30	432423	15	153722

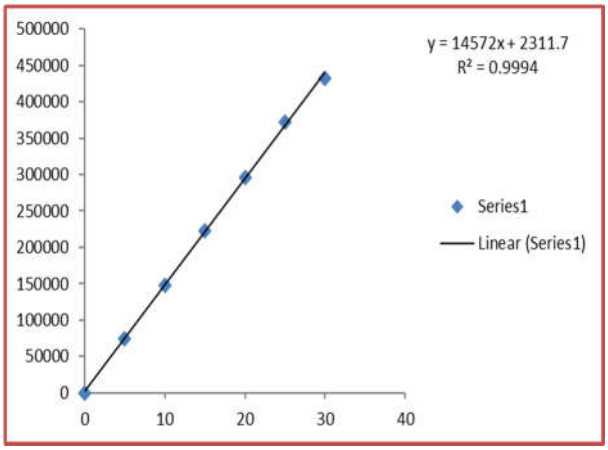

Fig 6: Calibration curve of Meloxicam

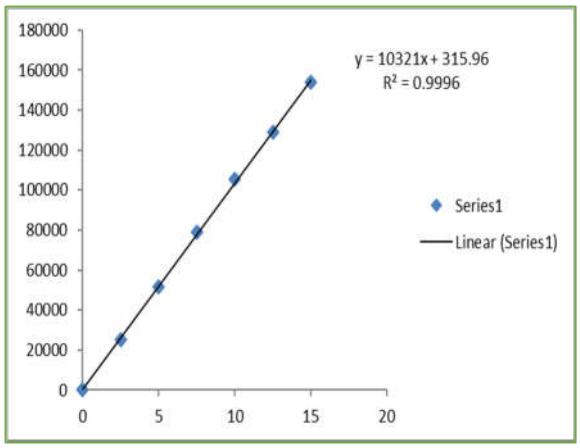

Fig 7: Calibration curve of Rizatriptan

Table 3: Regression data of Meloxicam and Rizatriptan

	Meloxicam	**Rizatriptan**
concentrations	5 – 30 µg/ml	2.5 – 15 µg/ml
Regression Co-efficient	y = 14572.x + 2311.7	y = 10321x + 315.96
Correlation Co-efficient	0.999	0.999

Precision:

Repeatability: Repeatability is the ability of a measurement system or process to produce consistent results when the same measurement is repeated multiple times under the same conditions. The repeatability was determined by injecting six standard solutions of 20µg/ml of Meloxicam and 10µg/ml of Rizatriptan. The %RSD was calculated which should be not more than 2%.

Table 4 : Repeatability results of Meloxicam and Rizatriptan

Injection	Meloxicam	Rizatriptan
Inj 1.	297326	105496
Inj 2.	296524	105269
Inj 3.	296332	105464
Inj 4.	297316	105236
Inj 5.	296865	106545
Inj 6.	296437	105260
Average	296800	105545
Std Dev	441.4	502.4
%.R.S.D	0.1	0.5

Intermediate Precision: Intermediate precision measures an analytical method's variability within a single laboratory over an extended period, accounting for changes in operators, equipment, days, and reagents. The intermediate precision was determined by injecting six standard solutions of 20µg/ml of Meloxicam and 10µg/ml of Rizatriptan. The %RSD was calculated which should be not more than 2%.

Table 5: Intermediate precision result of Meloxicam and Rizatriptan

Injection	Meloxicam	Rizatriptan
Inj 1.	294863	104086
Inj 2.	296698	103683
Inj 3.	292886	103854
Inj 4.	293582	103610
Inj 5.	293208	103384
Inj 6.	295465	104428
Average	294450.3	103841
Std Dev	1483.0	372.0
%.R.S.D	0.5	0.4

Accuracy: Analytical recovery tests were performed by using ICH guidelines to verify the recovery of a method that was developed and to study the interference of formulation excipients. The results of the recovery study and its statistical data are reveal in Table no. 6&7,

which shows that the designed technique has the highest accuracy. The recovery rate was 98.64% and 99.23% for Meloxicam and Rizatriptan respectively.

Table 6: Accuracy result of Meloxicam

% Level	Amount Taken	Amount reclaim	% Recovery	Mean %Recovery
50%	10	9.97	99.74	**99.64%**
		9.98	99.84	
		9.97	99.69	
100%	20	19.85	99.24	
		19.86	99.30	
		19.80	99.01	
150%	30	29.95	99.85	
		30.08	100.28	
		29.95	99.85	

Table 7: Accuracy result of Rizatriptan

% Level	Amount Taken	Amount reclaim	% Recovery	Mean %Recovery
50%	5	4.97	99.39	**99.23%**
	5	4.96	99.14	
	5	4.98	99.63	
100%	10	10.0	99.69	
	10	9.4	98.28	
	10	9.9	99.27	
150%	15	14.8	98.79	
	15	14.9	99.02	

	15	15.0	99.90	

Sensitivity: The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

Table 8: Sensitvity result of Meloxicam and Rizatriptan

Drug	LOD	LOQ
Meloxicam	0.08	0.24
Rizatriptan	0.06	0.17

Robustness: Robustness is the measure of a method remain unaffected by small deliberate changes in method parameters like conditions like Flow rate, mobile phase, and temperature. The samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Metformin, Dapagliflozin, and Saxagliptin

Condition	%R.S.D of Meloxicam	%R.S.D of Rizatriptan
FR (-) 0.9ml/min	0.4	0.2
FR (+) 1.1ml/min	0.4	0.3
MP (-) 35A:65B	0.5	0.2
MP (+) 45A:55B	0.3	0.4
T (-) 25°C	0.2	0.2
T (+) 35°C	0.2	0.5

Assay: The label for Symbravo states that it contains 20 mg of Meloxicam and 10 mg of Riztriptan. The aforementioned formulation was used for the assay. The average assay percentages for rizatriptan and meloxicam were 99.82 and 100.84, respectively.

Table 10: Assay of Meloxicam

Injection	Standard	Sample	% Assay
Inj 1.	296361	297326	99.99
Inj 2.	298526	296524	99.72
Inj 3.	295602	296332	99.66
Inj 4.	295901	297316	99.99
Inj 5.	296613	296865	99.83
Inj 6.	297566	296437	99.69
Average	296762	296800	99.81
Std Dev	1098.0	441.4	0.1484
%.R.S.D	0.4	0.1	0.15

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Table 11: Assay of Rizatriptan

Injection	Standard	Sample	% Assay
Inj 1.	105846	105496	99.67
Inj 2.	105134	105269	99.45
Inj 3.	105547	105464	99.64
Inj 4.	106108	105236	99.42
Inj 5.	105570	106545	100.66
Inj 6.	105605	105260	99.45
Average	105635	105545	99.7
Std Dev	326.2	502.4	0.47
%.R.S.D	0.3	0.5	0.48

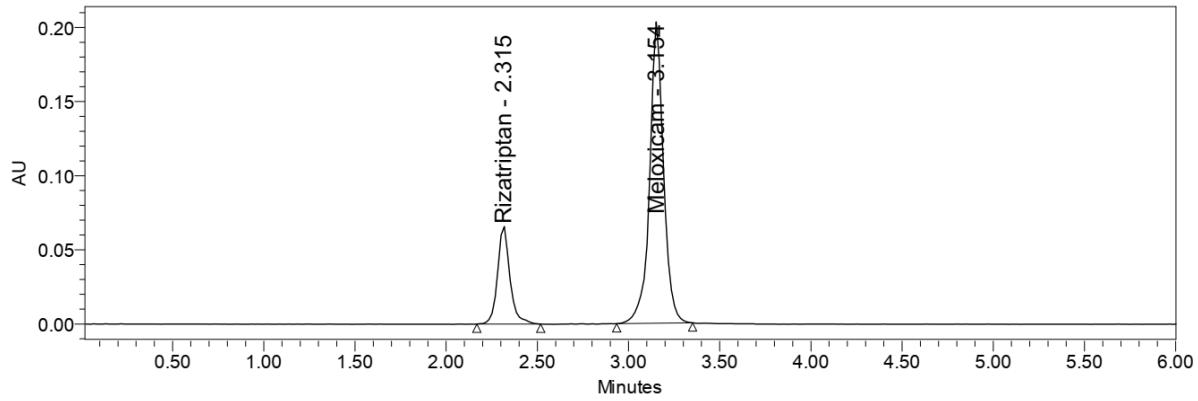

Fig 12: Standard solution Chromatogram

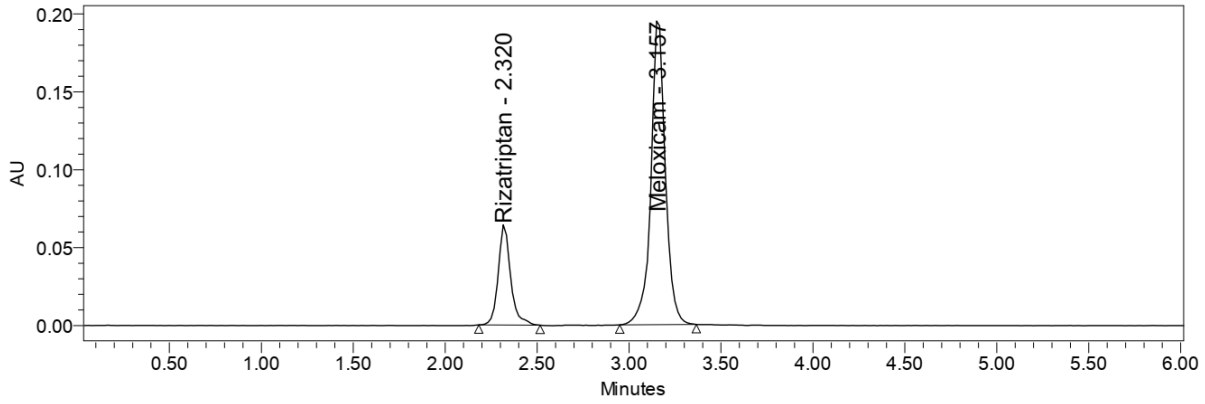

Fig 13: Sample solution Chromatogram

DEGRADATION

Degradation Studies:

Table 14: Degradation results of Meloxicam and Rizatriptan

Type of degradation	Meloxicam			Rizatriptan		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	278111	93.53	6.47	101238	95.65	4.35
Base	279234	93.91	6.09	101294	95.70	4.30
Peroxide	281594	94.70	5.30	101605	95.99	4.01
Thermal	289651	97.41	2.59	103593	97.87	2.13
Uv	291653	98.08	1.92	103851	98.11	1.89
Water	295348	99.32	0.68	104951	99.15	0.85

CONCLUSION

The present study successfully established a novel, precise, and stability-indicating RP-HPLC method for the simultaneous estimation of Meloxicam and Rizatriptan in bulk and pharmaceutical dosage forms. The chromatographic conditions were optimized to achieve sharp, symmetrical, and well-resolved peaks with short retention times, confirming the method's efficiency. Validation studies performed in accordance with ICH Q2 (R1) guidelines demonstrated excellent linearity, accuracy, precision, robustness, and specificity, with %RSD values below 2% and recovery within acceptable limits (98–102%). The low LOD and LOQ values indicated the method's high sensitivity.

The proposed method proved to be simple, rapid, cost-effective, and reliable, making it highly suitable for routine quality control, stability testing, and assay analysis of Meloxicam and Rizatriptan in combined formulations. Furthermore, the method can be extended for bioanalytical or dissolution studies with minor modifications, contributing to improved analytical quality assurance in pharmaceutical research.

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