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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CELECOXIB AND METFORMIN HYDROCHLORIDE IN SYNTHETIC MIXTURE

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ABSTRACT

The Objective of this study was to develop and validate precise, accurate, and rapid RP-HPLC method for the simultaneous estimation of Celecoxib and Metformin Hydrochloride in a synthetic mixture as per ICH guidelines. In RP-HPLC method, the chromatographic system was equipped with Kromstar C18 (250 mm × 4.6 mm internal diameter × 5 µm particle size). and UV detector set at 231 nm and a Mobile Phase consisting of Phosphate buffer: Methanol: Acetonitrile (pH: 2.8 adjusted with 10% Ortho phosphoric acid) (52:28:20 % v/v/v) was used at a flow rate 1 ml/min. The retention time was found to be approximately 2.5 min for Metformin and 5.8 min for Celecoxib, ensuring proper separation of both drugs. The various analytical parameters, including specificity, linearity, LOD, LOQ, precision, accuracy, and robustness, were determined ICH guidelines. RP-HPLC method showed good linearity, precision, and accuracy with correlation coefficients close to 1. Recovery studies were within acceptable limits. Low LOD and LOQ values indicated high sensitivity. Percentage assay values were close to 100%, confirming the reliability of the developed method. The developed RP-HPLC method was found to be specific, accurate, precise, robust, economical, and reproducible. This method is suitable for routine quality control analysis of Celecoxib and Metformin Hydrochloride in synthetic mixtures.

KEYWORDS: Celecoxib, Metformin Hydrochloride, Synthetic Mixture, RP-HPLC Method Development and Validation.

INTRODUCTION

Inflammation is a protective response of the body against harmful stimuli; however, persistent inflammation is associated with various chronic diseases. Non-steroidal anti-inflammatory drugs (NSAIDs), especially selective COX-2 inhibitors like celecoxib, are widely used due to their improved safety profile. Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin secretion or action, leading to serious complications if not properly managed. Metformin hydrochloride, a biguanide class drug, is commonly prescribed as first-line therapy due to its effectiveness in reducing hepatic glucose production and improving insulin sensitivity. Recent studies suggest a link between inflammation, metabolic disorders, and conditions such as familial adenomatous polyposis, where both celecoxib and metformin have shown potential therapeutic benefits. Therefore, the combination of these drugs may provide synergistic effects. Analytical method such as HPLC offers higher sensitivity and specificity. However, limited methods are available for the simultaneous estimation of celecoxib and metformin hydrochloride. Hence, the present study aims to develop and validate a simple, accurate, and precise analytical method for their simultaneous estimation in synthetic mixture.

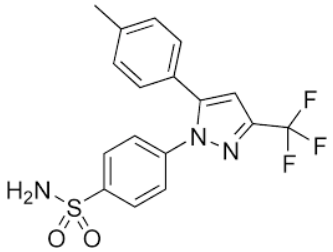

Figure 1: Structure of Celecoxib.

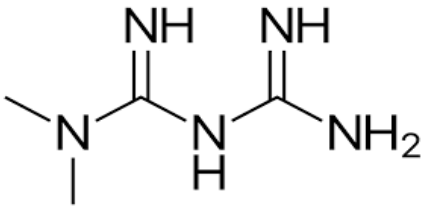

Figure 2: Structure of Metformin.

METHOD AND MATERIALS

Systronic RP-HPLC (LC-20-AD) (SPD-20 A) Instrument was used. UV Visible Spectrophotometer: Shimadzu model 1900 was used. Kromstar C18 (250 mm × 4.6 mm internal diameter × 5 µm particle size). API was obtained as gift sample Celecoxib from Zydus Pharmaceuticals Ltd., Ahmedabad and Metformin HCl from Deep Pharmaceuticals Ltd., Ahmedabad). Acetonitrile, Methanol, Water (HPLC grade) from Finar Chemicals Pvt. Ltd., India and Ortho Phosphoric Acid (AR Grade) from Deep Pharmaceuticals Ltd., Ahmedabad.

EXPERIMENTAL WORK

SELECTION OF MOBILE PHASE: Various mobile phases were tried. Trial contains various mobile phases which consisted of Acetonitrile, Methanol, Water, Phosphate buffer in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. Chromatogram in optimized mobile phase is shown in Figure.

Preparation of Mobile phase

Preparation of 10% Orthophosphoric acid 10% ortho phosphoric acid was prepared by diluting 1.0 ml of concentrated ortho phosphoric acid in 10 ml HPLC grade water.

Preparation of buffer (10 mM phosphate buffer) Accurately weighed 0.272 gm potassium dihydrogen phosphate (KH2PO4) was transferred it in 200 ml HPLC grade water and allowed it to dissolve. It was filtered through 0.45 µm membrane filter and sonicated for about 10 min. Buffer pH was adjusted to 4.0 with 10% ortho phosphoric acid.

Preparation of Mobile phase: Phosphate buffer: Methanol: Acetonitrile (pH: 2.8 adjusted with 10% ortho phosphoric acid) (52:28:20% v/v/v) Mobile phase was used after filtered it through 0.45 µm membrane filter and sonication.

Preparation of Standard stock solution

Celecoxib (40 µg/ml)

Accurately weighed Celecoxib (4 mg) was transferred to a 100 ml volumetric flask, and diluted up to the mark with mobile phase to obtain a standard stock solution (40 µg/ml).

Metformin HCl (100 µg/ml)

Accurately weighed Metformin HCl (10 mg) was transferred to a 100 ml volumetric flask, and diluted up to the mark with mobile phase to obtain a standard stock solution (100 µg/ml).

Preparation and analysis of synthetic mixture

The synthetic mixture of Celecoxib and Metformin HCl was prepared in the ratio of 400:1000. Accurately weighed equivalently weight of Celecoxib (400 mg) and Metformin HCl (1000 mg) and transferred in 100 ml volumetric flask and allow to sonicate and made up to mark with Methanol. Common excipients such as MCC (Micro Crystalline Cellulose) (8 mg), Lactose Monohydrate (70 mg), Polyvinyl Pyrrolidine (18 mg), Talc (2 mg) and Magnesium Stearate (2 mg) were added in the motor pestle along with the drug Celecoxib (400 mg) and Metformin (1000 mg). This solution was filtered through Whatmann filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 4000 µg/ml of Celecoxib and 10000 µg/ml of Metformin HCl. From above synthetic mixture solution, accurately 0.02 ml of the above mixture solution of (Celecoxib 4000 µg/ml and Metformin HCl 10000 µg/ml) was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Celecoxib was 8 µg/ml and Metformin HCl 20 µg/ml.

Table 1: Optimized Chromatographic condition.

Parameters	Chromatographic Condition
Column	Kromstar C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Phosphate buffer: Methanol: Acetonitrile (pH: 2.8 adjusted with 10% ortho phosphoric acid) (52:28:20% v/v/v)
Flow rate	1 ml/min
Run time	10 min
Detection wavelength	231 nm
Detector	Detector: U.V Detector
Injection volume	20 µL
Syringe	Hamilton

Method Validation

a. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.

b. Linearity & Range

The linearity of Celecoxib and Metformin HCl was found to be in the range of 4-20 µg/ml and 10-50 µg/ml, respectively. Plot the calibration curve of Peak area Vs Concentration (µg/ml). Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

c. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, Reproducibility (Interday precision), Repeatability.

d. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection can be calculated using following equation as per ICH guidelines.

$$LOD = 3.3 \times (\sigma / S)$$

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$LOQ = 10 \times (\sigma / S)$$

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

e. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50%, 100%, 150% and the values were measured for Celecoxib (8 µg/ml) and Metformin HCl (20 µg/ml). This performance was done in triplicate.

f. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

It should show the reliability of an analysis with respect to deliberate variation in method parameter.

RESULT AND DISCUSSION

METHOD DEVELOPMENT

Optimized Chromatogram

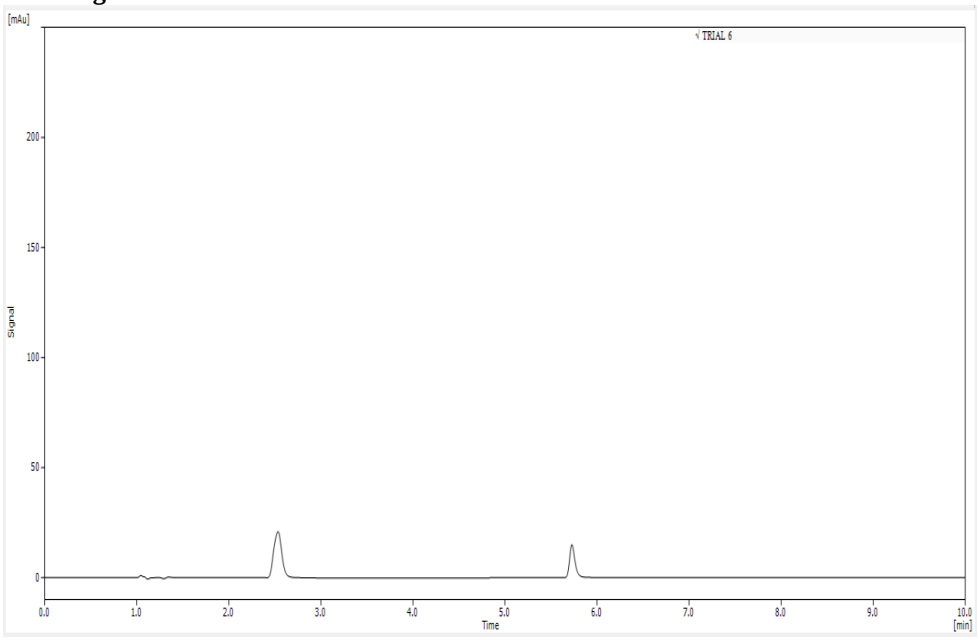

Figure 3: RP-HPLC Chromatogram of Celecoxib (8 µg/ml) and Metformin HCl (20 µg/ml) in Phosphate buffer: Methanol: Acetonitrile (pH: 2.8 adjusted with 10% ortho phosphoric acid) (52:28:20% v/v/v) at 231 nm {Run time: 10 min, Flow rate: 1 ml/min}.

METHOD VALIDATION

Specificity

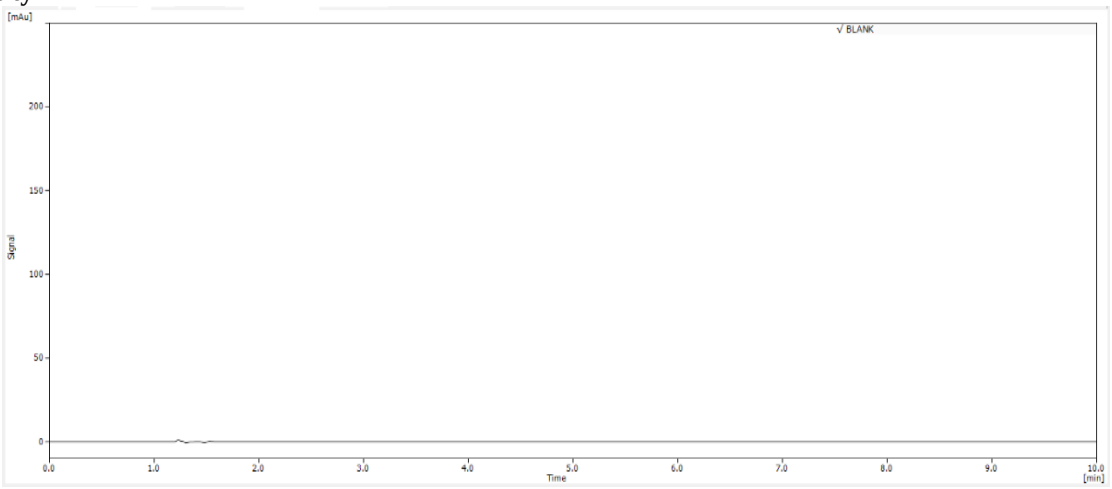

Figure 4: RP-HPLC Chromatogram of Blank in Phosphate buffer: Methanol: Acetonitrile (pH: 2.8 adjusted with 10% ortho phosphoric acid) (52:28:20% v/v/v) at 231 nm {Run time: 10 min, Flow rate: 1 ml/min}.

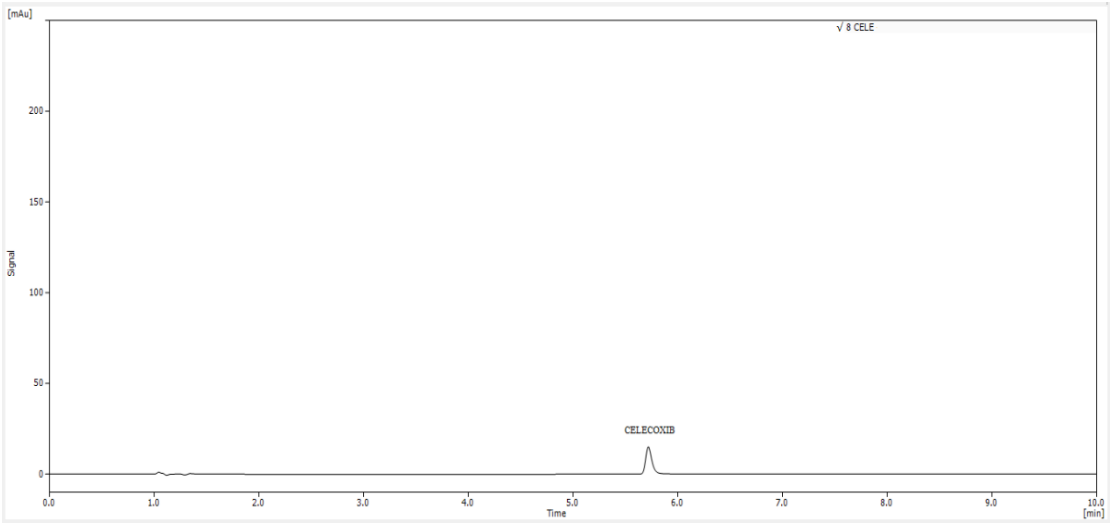

Figure 5: RP-HPLC Chromatogram of Celecoxib (8 µg/ml) in Phosphate buffer: Methanol: Acetonitrile (pH: 2.8 adjusted with 10% ortho phosphoric acid) (52:28:20% v/v/v) at 231 nm {Run time: 10 min, Flow rate: 1 ml/min}.

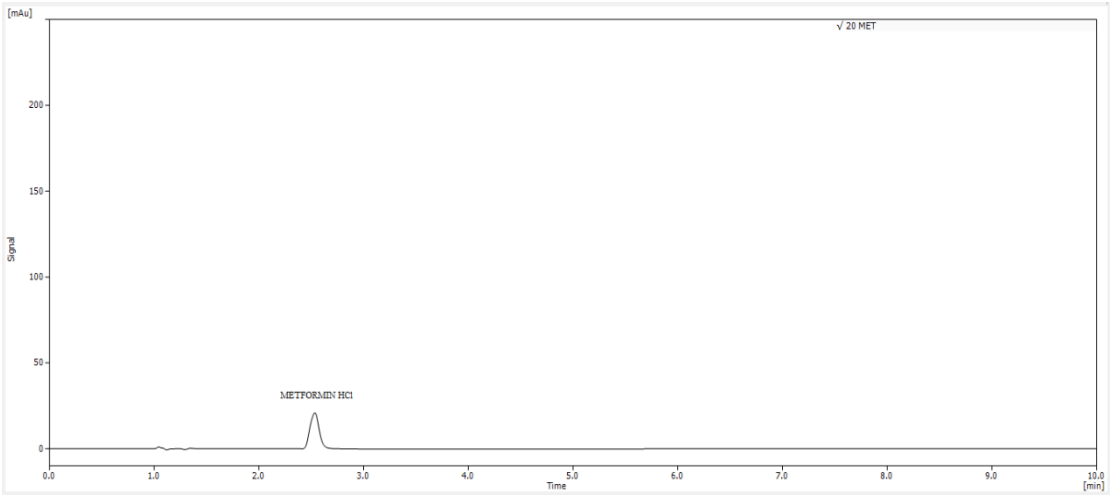

Figure 6: RP-HPLC Chromatogram of Metformin HCl (20 µg/ml) in Phosphate buffer: Methanol: Acetonitrile (pH: 2.8 adjusted with 10% ortho phosphoric acid) (52:28:20% v/v/v) at 231 nm {Run time: 10 min, Flow rate: 1 ml/min}.

Linearity

Table 2: Calibration data for Celecoxib (4-20 µg/ml) and Metformin HCl (10-50 µg/ml).

Sr. No	Concentration (µg/ml)		Mean Peak area (mAu*sec) ± S. D. (n=6)		% RSD	
	CELE	MET	CELE	MET	CELE	MET
1	4	10	25.754±0.3464	76.622±1.1180	1.35	1.46
2	8	20	136.249±1.6631	282.28±3.3772	1.22	1.20
3	12	30	263.396±2.8087	491.10±4.6696	1.07	0.95
4	16	40	376.82±3.1981	706.85±3.9499	0.85	0.56
5	20	50	496.43±2.9861	916.97±2.9814	0.60	0.33

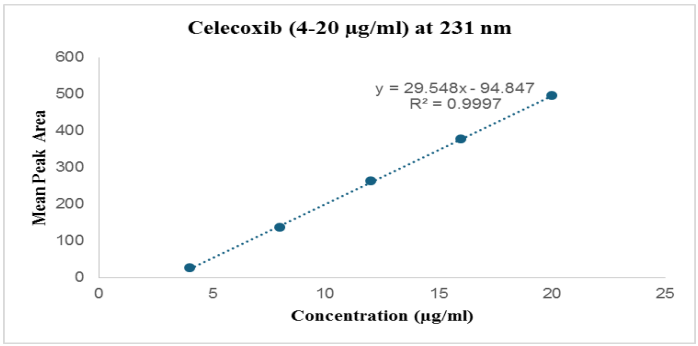

Figure 7: Calibration curve of Celecoxib (1-5 µg/ml) at 231 nm.

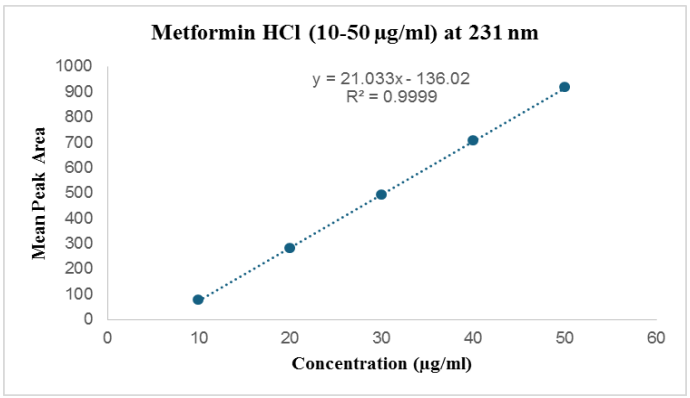

Figure 8: Calibration curve of Metformin HCl (10-50 µg/ml) at 231 nm.

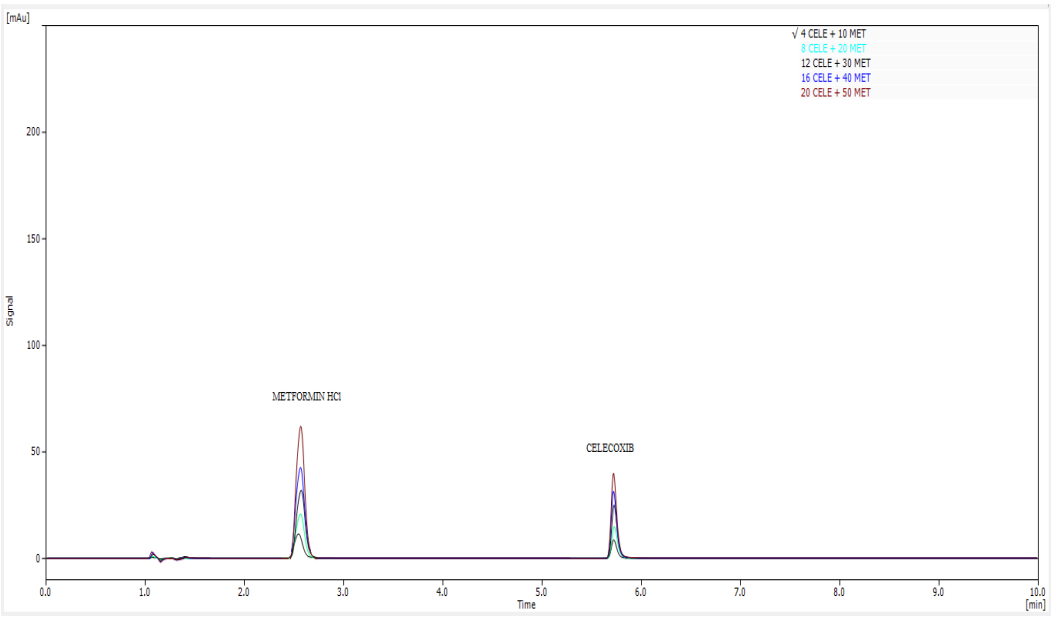

Figure 9: Overlain RP-HPLC chromatogram of Celecoxib (4-20 µg/ml) and Metformin HCl (10-50 µg/ml) at 231 nm {Run time: 10 min, Flow rate: 1 ml/min}.

Table 3: Precision study for Celecoxib.

Celecoxib at 231 nm		
Intraday precision of Celecoxib		
Conc. (µg/ml)	**Mean peak area (mAu*sec) ± S.D (n=3)**	**% RSD**
4	25.620±0.3399	1.33
8	134.616±1.6540	1.23
12	261.596±2.5456	0.97
Interday precision of Celecoxib		
Conc. (µg/ml)	**Mean peak area (mAu *sec) ± S.D (n=3)**	**% RSD**
4	25.654±0.3559	1.39
8	134.582±1.6997	1.26
12	261.396±2.8284	1.08
Repeatability of Celecoxib		
Conc. (µg/ml)	**Mean peak area (mAu *sec) ±SD (n=6)**	**% RSD**
8	136.266±1.6499	1.21

Table 4: Precision study for Metformin HCl.

Metformin HCl at 231 nm		
Intraday precision of Metformin HCl		
Conc. (µg/ml)	**Mean peak area (mAu*sec) ±SD (n=3)**	**% RSD**
10	76.688±1.1441	1.49
20	285.212±3.3536	1.18
30	493.430±4.4907	0.91
Interday precision of Metformin HCl		
Conc. (µg/ml)	**Mean peak area (mAu *sec) ±SD (n=3)**	**% RSD**
10	76.722±1.1576	1.51
20	285.250±3.3993	1.19
30	493.100±4.9046	0.99
Repeatability of Metformin HCl		
Conc. (µg/ml)	**Mean peak area (mAu *sec) ±SD (n=6)**	**% RSD**
20	282.450±3.2796	1.16

Table 5: Recovery study data.

Name of Drug	% Level of Recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Spiked Std Amount (µg/ml)	Total amount Recovered (µg/ml)	% Recovery ±S. D (n=3)
Celecoxib	50	8	4	12	11.987	99.89±1.1325
	100	8	8	16	15.989	99.93±1.0643
	150	8	12	20	19.994	99.97±0.8361
Metformin HCl	50	20	10	30	29.976	99.92±0.9652
	100	20	20	40	39.98	99.95±0.6148
	150	20	30	50	49.99	99.98±0.3433

Table 6: LOD and LOQ data.

Drug Name	Celecoxib	Metformin HCl
LOD (µg/ml)	0.04	0.18
LOQ (µg/ml)	0.12	0.53

Table 7: Analysis of synthetic mixture.

Drug Name	Amount in synthetic mixture (µg/ml)	Amount found (µg/ml)	% Assay ± S.D. (n=3)
Celecoxib	8	7.988	99.85±0.8146
Metformin HCl	20	19.992	99.96±0.7042

Table 8: Robustness data.

Condition	Variation	Celecoxib	Metformin HCl
		% Assay ± SD (n=3)	% Assay ± SD (n=3)
Flow rate (1 ml ± 0.2 ml/ min)	0.8 ml/min	97.75±2.3730	98.94±4.5166
	1.0 ml/min	99.75±3.5545	99.67±4.2691
	1.2 ml/min	97.25±5.0286	98.82±6.4770
Detection wavelength (231 nm ± 2 nm)	229	97.25±0.9454	99.65±6.1268
	231	100.25±1.5055	100.01±6.4267
	233	98.75±1.3762	99.53±7.0256
Mobile Phase Phosphate buffer: Methanol: Acetonitrile (pH:2.8) (52:28:20 ± 2 % v/v/v)	50:26:24	97.75±2.1116	99.26±2.0784
	52:28:20	99.75±2.0552	99.85±1.9421
	48:30:22	98.25±2.1845	98.95±2.0143

Table 9: Summary of Validation Parameters.

Sr. No.	Parameters	Celecoxib	Metformin HCl
1	Linearity Range (µg/ml)	4-20	10-50
2	Regression equation (y = mx +c)	y = 29.548x-94.847	y = 21.033x - 136.02
3	Correlation Coefficient (r^2)	0.9997	0.9999
4	Intraday Precision (%RSD, n=3)	0.97-1.33	0.91-1.49
5	Interday Precision (% RSD, n=3)	1.08-1.39	0.99-1.51
6	Repeatability (% RSD, n=6)	1.21	1.16
7	Accuracy (% Recovery, n=3)	99.89%-99.97%	99.92%-99.98%
8	LOD (µg/ml)	0.04	0.18
9	LOQ (µg/ml)	0.12	0.53
10	% Assay	99.85%	99.96%

CONCLUSION

A rapid, sensitive, accurate and precise RP-HPLC method has been developed and validated for routine analysis of Celecoxib and Metformin HCl in Synthetic mixture. The RP-HPLC method is suitable for simultaneous estimation of Celecoxib and Metformin HCl in Synthetic mixture in without interference of each other. The developed method was successfully applied in Synthetic mixture. The proposed method can be utilized for the routine analysis of Celecoxib and Metformin HCl in Synthetic mixture.

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