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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF CELECOXIB AND TRAMADOL HYDROCHLORIDE

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ABSTRACT
An analytical simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Celecoxib and Tramadol HCL in bulk and marketed pharmaceutical dosage forms. This Separation was carried out on Symmetry C_{18} (250 x 4.6mm, 5µm particle size) column in isocratic mode with mobile phase containing Methanol and Phosphate Buffer were taken in proportion of 60:40% v/v adjusted to pH 3.6 using ortho phosphoric acid. The flow rate was 1.0 ml/min and effluent was monitored at 330 nm. The retention times for Celecoxib and Tramadol HCL were 2.131 and 3.056 min respectively. The method is useful in the quality control of bulk and pharmaceutical formulations. The method was validated for accuracy, precision, linearity, robustness, ruggedness and LOD & LOQ of standard solution. The developed method was found to be accurate, precise and selective for simultaneous determination of Celecoxib and Tramadol HCL in bulk and marketed pharmaceutical dosage forms.

KEYWORDS: Celecoxib and Tramadol HCL, RP-HPLC, Accuracy, Precision.

INTRODUCTION
Celecoxib is a member of the class of pyrazoles that is 1H-pyrazole which is substituted at positions 1, 3 and 5 by 4-sulfamoylphenyl, trifluoromethyl and p-tolyl groups, respectively. A cyclooxygenase-2 inhibitor, it is used in the treatment of arthritis. It has a role as a cyclooxygenase 2 inhibitor, a geroprotector, a non-steroidal anti-inflammatory drug and a non-narcotic analgesic.[1] It is a member of toluenes, a sulfonamide, a member of pyrazoles and an organofluorine compound. Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, is a nonsteroidal anti-inflammatory drug (NSAID) which is known for its decreased risk of causing gastrointestinal bleeding compared to other NSAIDS. It is used to manage symptoms of various types of arthritis pain and in familial adenomatous polyposis (FAP) to reduce precancerous polyps in the colon.[2] It is marketed by Pfizer under the brand name Celebrex, and was initially granted FDA approval in 1998. Interestingly, selective COX-2 inhibitors (especially celecoxib), have been evaluated as potential cancer chemopreventive and therapeutic drugs in clinical trials for a variety of malignancies.[3] Celecoxib is a Nonsteroidal Anti-inflammatory Drug. The mechanism of action of celecoxib is as a Cyclooxygenase Inhibitor. The IUPAC Name of 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzene sulfonamide. The Chemical Structure of Celecoxib is shown in following figure-1.

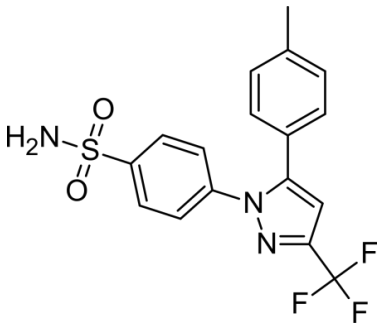

Fig. 1: Chemical Structure of Celecoxib

Tramadol, sold under the brand name Ultram among others, is an opioid pain medication and a serotonin–norepinephrine reuptake inhibitor (SNRI) used to treat moderately severe pain. When taken by mouth in an

immediate-release formulation, the onset of pain relief usually begins within an hour. It is also available by injection. It is available in combination with paracetamol (acetaminophen).[4] As is typical of opioids, common side effects include constipation, itchiness, and nausea. Serious side effects may include hallucinations, seizures, and increased risk of serotonin syndrome, decreased alertness, and drug addiction. A change in dosage may be recommended in those with kidney or liver problems.[5] It is not recommended in those who are at risk of suicide or in those who are pregnant. While not recommended in women who are breastfeeding, those who take a single dose should not generally have to stop breastfeeding.[6] Tramadol is converted in the liver to O-desmethyl tramadol (desmetramadol), an opioid with a stronger affinity for the μ-opioid receptor. Tramadol is an Opioid Agonist.[7] The mechanism of action of tramadol is as a Full Opioid Agonist. The IUPAC name of Tramadol HCL is (1R, 2R)-2-[(dimethyl amino) methyl]-1-(3-methoxy phenyl) cyclohexan-1-ol; hydrochloride. The Chemical Structure of Tramadol HCL is shown in follows

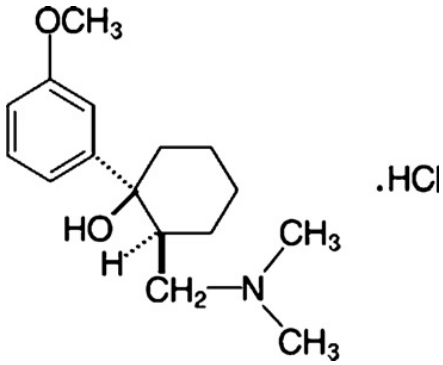

Fig. 2: Chemical Structure of Tramadol HCL.

A literature survey [36-40] reveals that analytical methods based on HPLC, HPTLC, UV Spectrometry are available for the determination of this drug individually and in combination with other drugs in different dosage forms, there is one analytical method reported with Methanol and Phosphate Buffer in the ratio of 60:40% v/v with pH 3.6 for the simultaneous estimation of Celecoxib and Tramadol HCL in a bulk and Combined Dosage Form. The aim of the present work is develop a simple, precise, accurate, and rapid method with less run time for the determination of Celecoxib and Tramadol HCL in a bulk form and Pharmaceutical Combined Dosage Form without lack of interference.

MATERIALS AND METHODS

Instruments Used:

Table-1: Instruments Used

Sr. no.	Name of Instrument	Instrument Model	Name of Manufacturer
1	UV-Visible Double Beam Spectrophotometer	UV 1800	Elico
2	HPLC	717	Waters
3	Ultra Sonicator	--------	Entrech Electronics Limited
4	Vaccum filtration kit	---------	Labindia
5	pH Meter	pH-7000	Labindia

Chemicals / Reagents Used

Table-2: Chemicals Used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Potassium dihydrogen ortho phosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Hydrochloric acid	99.9%	A.R.	A.R Chemicals Pvt.Ltd
6.	Sodium Hydroxide	99.9%	A.R.	A.R Chemicals Pvt.Ltd
7.	3% Hydrogen Peroxide	99.9%	A.R.	A.R Chemicals Pvt.Ltd

UV analysis for Development of Method and validation of developed method for Simultaneous determination of Celecoxib and Tramadol HCL

Preparation of Standard Stock Solution of Celecoxib

Accurately weighed 10mg of Celecoxib and it was transferred to clean and dry 100 ml of volumetric flask and dissolved in Methanol : buffer (60:40% v/v) and made-up the volume to 100 ml with same solvent system.[8] The final solution contained 100μg per ml of Celecoxib solution.

Preparation of Standard Stock Solution of Tramadol HCL

Accurately weighed Tramadol HCL (10mg) was transferred to 100ml volumetric flask, dissolved in Methanol: buffer (60:40% v/v) and made-up the volume to 100 ml with same solvent system. The final solution contained 100 μg per ml of Tramadol HCL solution.

Determination of Wavelength of Maximum Absorbance for Celecoxib

Standard Celecoxib solution (1ml) was transferred to separate 10 ml volumetric flask. The final volume was adjusted to 10 ml with the same mobile phase. The absorbance of the final resulted solution was scanned in the range 200 to 400 nm against mobile phase as blank.[9]

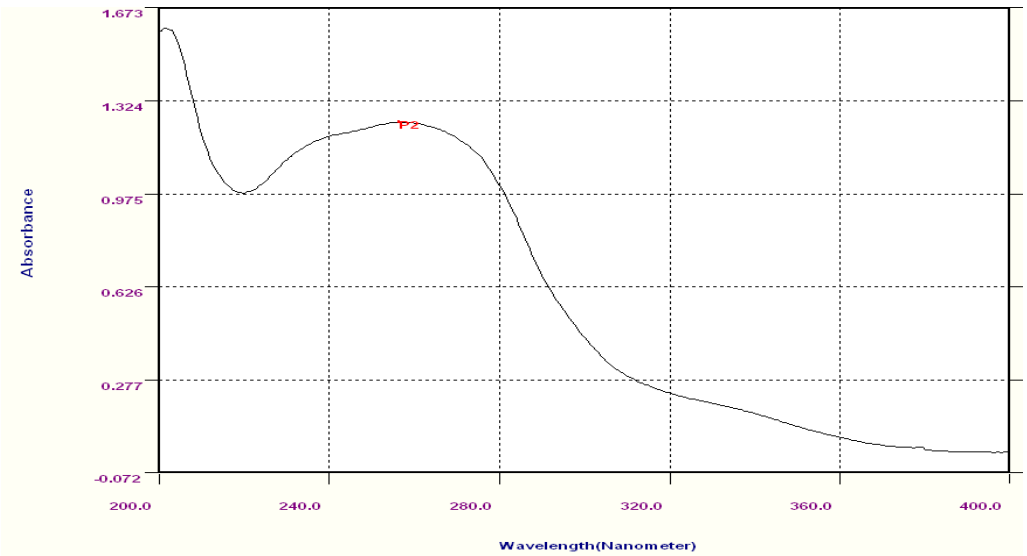

Fig. 3: UV Spectrum of Celecoxib (261 nm).

Estimation of Maximum Wavelength for Tramadol HCL

First of all take 1ml of standard Tramadol HCL solution from the above standard solution (1 ml) was transferred to separate clean and dry of 10 ml volumetric flask. The final volume was adjusted to 10ml with same mobile phase (Solvent). The absorbance of the final resulted solution was scanned in the range 200 to 400 nm against solvent mixture as blank. The results are shown in following figure-4.

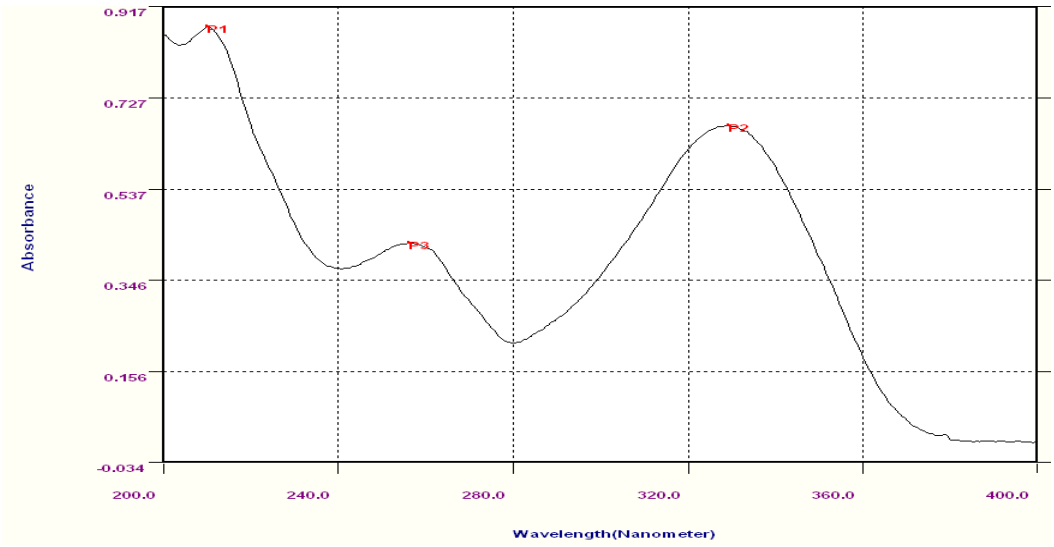

Fig. 4: UV Spectrum of Tramadol HCL (330 nm).

Method Development for RP-HPLC

Selection of Wavelength

The λ_{max} of the two ingredients i.e. Celecoxib and Tramadol HCL, were found to be 261 nm and 330 nm respectively in Methanol and Phosphate Buffer (pH-3.6) (60:40% v/v) as solvent system. As two drugs having almost near absorption max & at 261 nm Celecoxib shows more intense as compare to Tramadol HCL at 330 nm, 330 nm has been chosen as common absorption maximum for RP-HPLC analysis.[10]

Preparation of Standard Solution of Celecoxib

Weighed accurately 10mg of standard Celecoxib and transferred into clean & dry 100 ml volumetric flask. Then 20 ml of mobile phase was added and sonicated to dissolve in 100ml of volumetric flask. The final volume was made up to the mark with same solvent. The final solution contained about 10µg/ml of Celecoxib.

Preparation of Standard Solution of Tramadol HCL
First 10 mg of Tramadol HCL was weighed accurately and transferred into clean & dry 100 ml volumetric flask. Then 20 ml of mobile phase was added and sonicated to dissolve it in mobile phase. The final volume was made up to the mark with same solvent. The final solution contained about 10µg/ml of Tramadol HCL.

Initialization of the Instrument
The HPLC instrument was switched on. First the column was washed with the HPLC grade water for 45 minutes. After washing the column that the column is saturated with the mobile phase in 45 minutes. The mobile phase was run to find the peaks or identification of peaks.[11] After 20 minutes the standard drug solution was prepared and injected in HPLC system.

Preparation of Mobile Phase
The mobile phase can be prepared by taking Methanol: Phosphate Buffer and maintained pH-3.6 with diluted orthophosphoric acid (60:40% v/v). The resulted Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath.[12] The final obtained mobile phase was pumped through the selected column and maintained at a flow rate of 1.0 ml/min.

Preparation of API Mixtures of Celecoxib and Tramadol HCL
The Celecoxib and Tramadol HCL API mixtures were prepared in ratio of 1:1 and stock solution prepared as described in section (Preparation of Standard Solution of Celecoxib) and (Preparation of Standard Solution of Tramadol HCL). The resultant solution was filtered through a 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. From the above resulting solution several working standard solutions were prepared by using serial dilution technique.[13]

Running the API Mixture of Celecoxib and Tramadol HCL
1 ml of stock solution (100ppm) was pipetted out into 10 ml volumetric flask and volume was made up to the mark with the mobile phase (Solvent). The final solution was filtered through a 0.45 µm efficient membrane filter and degassed under ultrasonic bath. The resulted solution was injected into the HPLC system. The chromatogram obtained is shown in following figure-5.

Different Chromatographic Conditions Used and Their Optimizations
The various HPLC chromatographic conditions are used to fin the optimum chromatographic condition for best elution of drugs in the mixture.

Method Validation by RP-HPLC
System Suitability
As per the test method, the standard solutions were prepared and injected into HPLC system from which the evaluated system suitability parameters are found to be within the limits.[14]

Linearity
The ability of the method to produce results those are directly or indirectly proportional to the concentration of the analyst in samples within the limits.[15]

Precision
The degree of the closeness of agreement among individual test results when a method is applied to multiple samplings of a homogeneous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or repeatability (agreement under the same conditions) of the method.[16]

Accuracy
The closeness of results was obtained by a method to the true value. It is a measure of the exactness of the method.[17]

Limit of detection (LOD) and limit of quantification (LOQ)
The detection limit and quantification limit for each analyte were determined based on a signal-to-noise concept, as the lowest concentration at which signal-to-noise ratio between 3 or 2:1 and 10:1, respectively, with defined precision and accuracy under the given experimental conditions.[18]

Robustness
Robustness of the method was studied by slightly changes in experimental conditions such as flow rate and organic composition. Robustness on performed same instrument with different chromatic conditions.[19]

Ruggedness (Intermediate Precision)
Ruggedness of the method was studied using different source of analysts, instruments, and columns with same experimental conditions.[20]

Stability Studies
Following protocol was strictly adhered to for forced degradation of Celecoxib and Tramadol HCL Active Pharmaceutical Ingredient (API). The API (Celecoxib & Tramadol HCL) was subjected to kept in some stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. It is one type of accelerated stability studies of the drugs that is used to help us to determining the total fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing.[21] The different types of forced degradation pathways/studies are studied here are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation.

RESULTS AND DISCUSSION

Analytical Method Development

Optimization of Method

Table-3: Optimized Chromatographic Conditions

Mobile phase	Methanol : Phosphate Buffer pH-3.6 with OPA (60:40% v/v)
Wavelength	330 nm
Flow rate	1.0 ml/ min.
Injection Volume	20µl
Run time	7 min.
Column	Symmetry C_{18} (250 x 4.6mm, 5µm particle size)

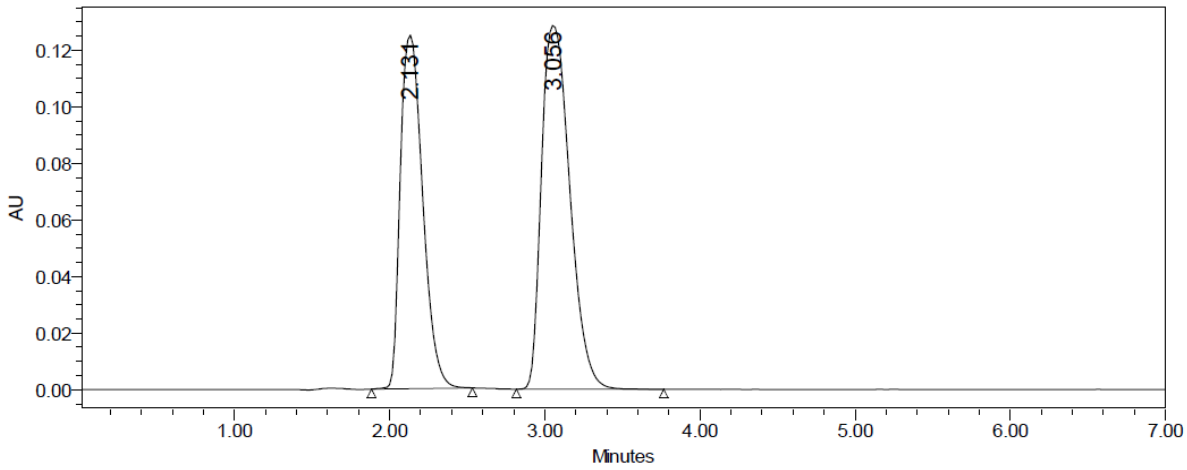

Fig. 5: Chromatogram for Optimized Chromatographic Condition.

Validation of Analytical Method:

In this method, system suitability, linearity, precision, accuracy, robustness, LOD, LOQ, and stability studies are validated for the selected Celecoxib and Tramadol HCL drugs.[22]

Linearity and Range

Standard solutions of Celecoxib in the concentration range of 0 µg/ml to 16 µg/ml were obtained by transferring (0.6, 0.8, 1.0, 1.2, 1.4, 1.6ml) of Celecoxib stock solution (100 µg/ml) to the series of 10 ml volumetric flasks and standard solutions of Tramadol HCL in the concentration range of 0 µg/ml to 16 µg/ml were obtained by transferring (0.6, 0.8, 1.0, 1.2, 1.4, 1.6ml) of Tramadol HCL stock solution (100 µg/ml) to the separate series of 10ml volumetric flasks. The volumetric flasks were made up to the mark with mobile phase. The solutions were filtered through a 0.45 µm membrane filter and degassed under ultrasonic bath. The final resulted solutions were injected into HPLC the system.[23] The run time maintained was 7 min and the various types of peak areas were measured.

Table 4: Calibration Data for Celecoxib.

S. No.	Conc. (µg/ml)	Peak Area
1	0	0
2	6	641233
3	8	844610
4	10	1052647
5	12	1250435
6	14	1465354
7	16	1662043

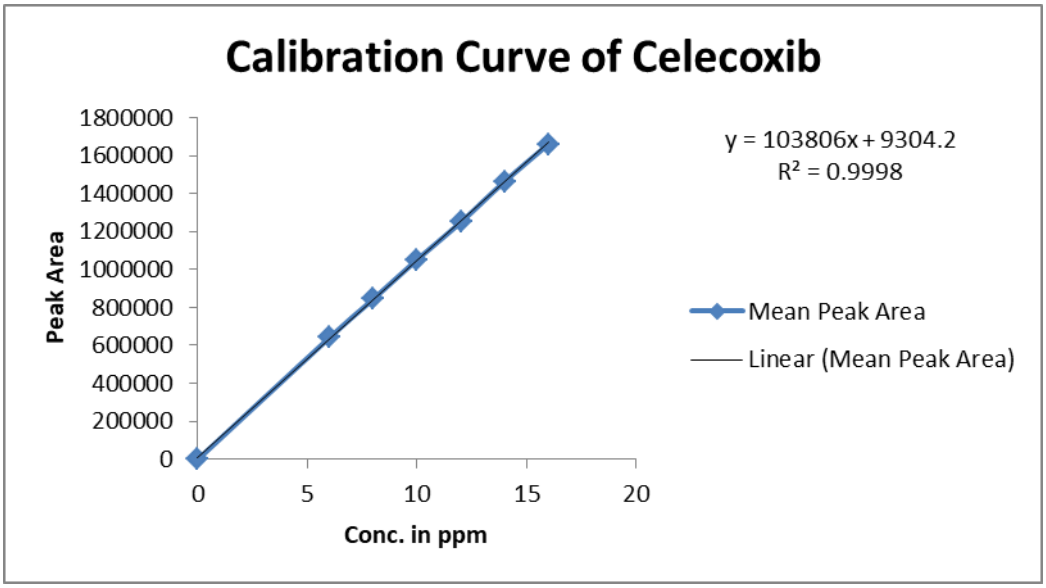

Fig. 6: Calibration Curve for Celecoxib.

Table-5: Calibration Data for Tramadol HCL

Sr. No.	Conc. (µg/ml)	Peak Area
1	0	0
2	6	628423
3	8	835412
4	10	1045742
5	12	1254033
6	14	1452471
7	16	1653504

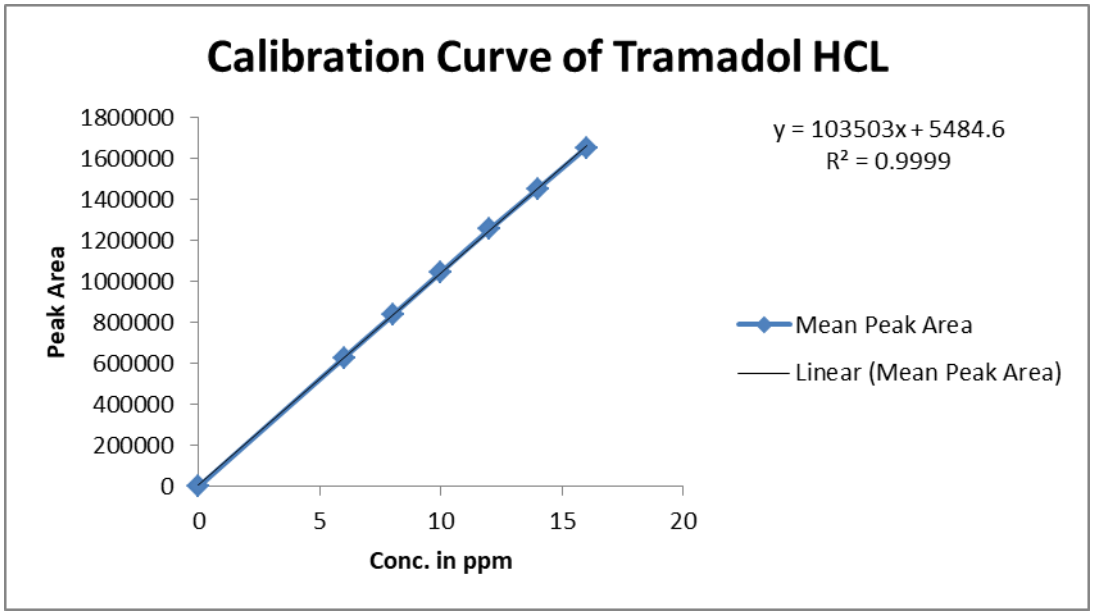

Fig-7: Calibration Curve for Tramadol HCL.

Observation: Linearity range was found to be 0-16 µg/ml for Celecoxib and 0-16 µg/ml for Tramadol HCL. The correlation coefficient was found to be 0.999 & 0.999 and the slope was found to be 10380 & 10350 and intercept were found to be 9304 & 5484 for Celecoxib and Tramadol HCL respectively.

Specificity

Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the mobile phase without drug.[24] Drug solutions were prepared individually and the sample and standard containing two

drugs was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific.

The chromatograms representing the peaks of blank, Celecoxib and Tramadol HCL Standard and the sample containing the two drugs were shown in following figures respectively.

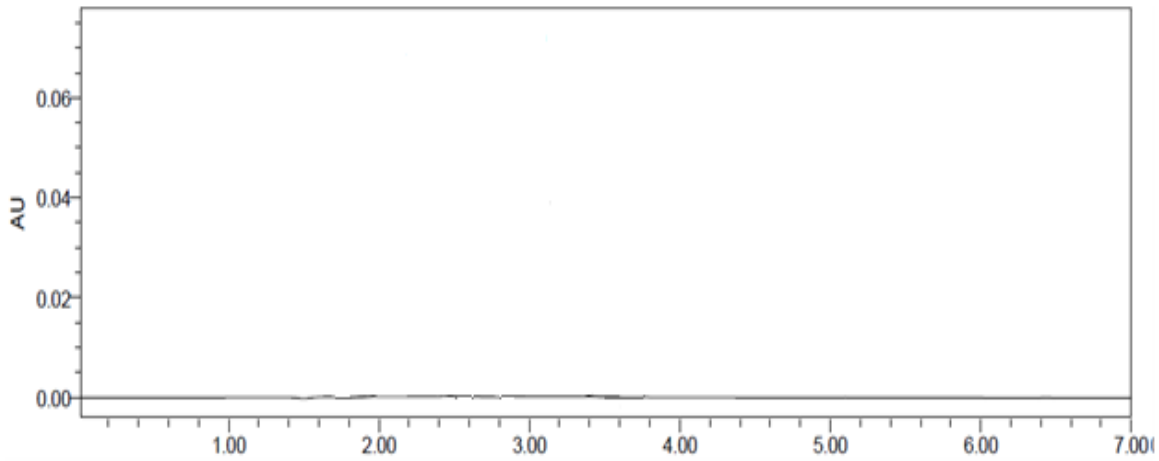

Fig. 8: Chromatogram of Blank Solution.

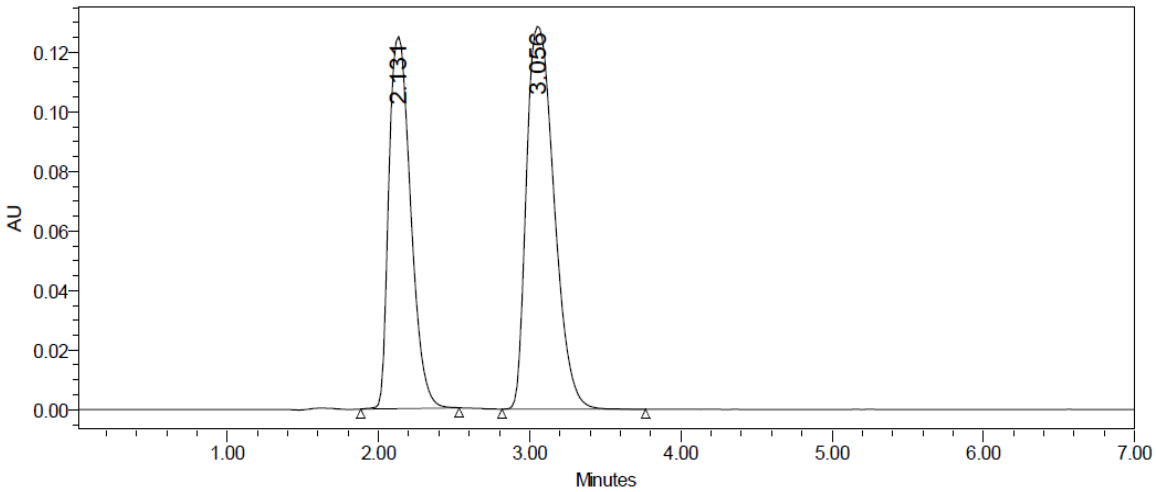

Fig. 9: Chromatogram of Celecoxib and Tramadol HCL Standard Solution.

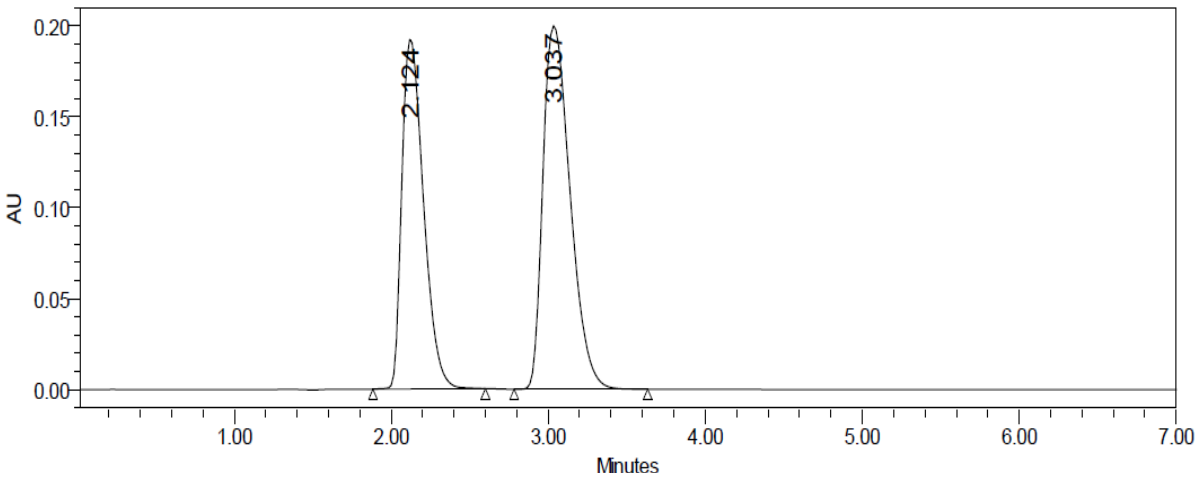

Fig. 10: Chromatogram of Celecoxib and Tramadol HCL Sample Solution.

Observation: In this test method blank, standard and sample solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

Accuracy:

Recovery Study of Celecoxib: The accuracy of the proposed developed method the % recovery studies were carried out by adding different quantities (80%, 100%, and 120%) of pure drug of CELECOXIB was taken and added to the prepared pre-analyzed formulation of concentration 10µg/ml.[25] From that % recovery values were measured. The results were shown in Table-6.

Table-6: Data of Recovery Studies for Celecoxib.

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.105	93435	101.312	Mean= 100.0163% S.D. = 1.293505 % R.S.D.= 1.293294
S_2 : 80 %	8	7.898	91287	98.725	
S_3 : 80 %	8	8.001	92356	100.012	
S_4 : 100 %	10	10.195	115135	101.95	Mean= 101.4033% S.D. = 0.613379 % R.S.D.= 0.60489
S_5 : 100 %	10	10.152	114687	101.52	
S_6 : 100 %	10	10.074	113879	100.74	
S_7 : 120 %	12	12.171	135647	101.425	Mean= 100.6053% S.D. = 0.730041 % R.S.D. = 0.725649
S_8 : 120 %	12	12.044	134324	100.366	
S_9 : 120 %	12	12.003	133897	100.025	

Accuracy

Recovery Study of Tramadol HCL: To determine the accuracy of the given developed method the percentage recovery studies were carried out by adding different quantities (80%, 100%, and 120%) of pure drug of Tramadol HCL was taken and added into the pre-analyzed formulation of concentration 10µg/ml.[26] From that % recovery values were determined. The results were shown in Table-7.

Table 7: Data of Recovery Studies for Tramadol HCL.

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.100	89325	101.25	Mean= 100.1207% S.D. = 1.251602 % R.S.D.= 1.250093
S_2 : 80 %	8	8.027	88569	100.337	
S_3 : 80 %	8	7.902	87279	98.775	
S_4 : 100 %	10	10.122	110254	101.22	Mean= 101.44% S.D. = 0.330454% R.S.D.= 0.325763
S_5 : 100 %	10	10.128	110312	101.28	
S_6 : 100 %	10	10.182	110874	101.82	
S_7 : 120 %	12	12.147	131215	101.225	Mean= 101.444% S.D. = 0.284828 % R.S.D. = 0.280774
S_8 : 120 %	12	12.161	131356	101.341	
S_9 : 120 %	12	12.212	131879	101.766	

Precision

Repeatability: Repeatability was assessed using six time repetition of working concentration.[27] The results are shown in Table-8 & 9.

Table 8: **Data Showing Repeatability Analysis for Celecoxib.**

HPLC Injection Replicates of Celecoxib	Peak Area
Replicate – 1	1013546
Replicate – 2	1025824
Replicate – 3	1012351
Replicate – 4	1036584
Replicate – 5	1015419
Replicate – 6	1008572
Average	**1018716**

Standard Deviation	**10495.73**
% RSD	**1.03029**

Table 9: **Data Showing Repeatability Analysis for Tramadol HCL.**

HPLC Injection Replicates of Tramadol HCL	**Peak Area**
Replicate – 1	1035681
Replicate – 2	1065897
Replicate – 3	1078953
Replicate – 4	1058748
Replicate – 5	1078754
Replicate – 6	1065871
Average	**1063984**
Standard Deviation	**15986.99**
% RSD	**1.50256**

Observation: The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml for Celecoxib and 10 µg/ml for Tramadol HCL (n =6) showed a RSD of 1.03029% for Celecoxib and 1.50256% for Tramadol HCL. It was concluded that the analytical technique showed good repeatability.

Intermediate Precision: Intermediate Precision was assessed using 6 replicate injections of working concentrations analyst 1 and analyst 2.[28] The results were shown in table-10, 11, 12 and 13.

Analyst-1:

Table-10: Results of Intermediate Precision Analyst 1 for Celecoxib.

S. No.	**Peak Name**	**RT**	**Area (µV*sec)**	**Theoretical Plates**	**Tailing Factor**
1	Celecoxib	2.131	1036584	3562	0.90
2	Celecoxib	2.136	1036582	3265	0.93
3	Celecoxib	2.134	1036985	3451	0.99
4	Celecoxib	2.132	1034587	3265	0.98
5	Celecoxib	2.131	1032859	3689	0.92
6	Celecoxib	2.134	1032548	3785	0.98
Mean			**1035024**		
Std. Dev.			**1985.712**		
% RSD			**0.191852**		

Table-11: Results of Intermediate Precision Analyst 1 for Tramadol HCL.

S.No.	**Peak Name**	**RT**	**Area (µV*sec)**	**Theoretical Plates**	**Tailing Factor**
1	Tramadol HCL	3.054	1052685	3633	1.20
2	Tramadol HCL	3.059	1058748	3658	1.18
3	Tramadol HCL	3059	1054213	3487	1.14
4	Tramadol HCL	3.055	1059685	3698	1.16
5	Tramadol HCL	3.056	1054178	3641	1.10
6	Tramadol HCL	3.059	1056398	3628	1.17
Mean			**1055985**		
Std. Dev.			**2785.318**		
% RSD			**0.263765**		

Analyst 2:

Table-12: Results of Intermediate Precision Analyst 2 for Celecoxib.

S.No.	**Peak Name**	**RT**	**Area (µV*sec)**	**Theoretical Plates**	**Tailing Factor**
1	Celecoxib	2.127	1045865	3354	0.99
2	Celecoxib	2.131	1045274	3362	0.97
3	Celecoxib	2.131	1047582	3385	0.98
4	Celecoxib	2.129	1047524	3392	0.96
5	Celecoxib	2.134	1046958	3396	0.98
6	Celecoxib	2.127	1047859	3374	0.93
Mean			**1046844**		

Std. Dev.			**1046.289**		
% RSD			**0.099947**		

Table-13: Results of Intermediate Precision Analyst 2 for Tramadol HCL.

S.No.	Peak Name	RT	Area (µV*sec)	Theoretical Plates	Tailing Factor
1	Tramadol HCL	3.050	1063599	3745	1.25
2	Tramadol HCL	3.058	1063598	3746	1.21
3	Tramadol HCL	3.055	1065471	3752	1.23
4	Tramadol HCL	3.049	1065285	3763	1.29
5	Tramadol HCL	3.056	1064574	3754	1.21
6	Tramadol HCL	3.038	1065478	3765	1.18
Mean			**1064668**		
Std. Dev.			**891.9746**		
% RSD			**0.08378**		

Observation: The intraday and interday studies results show that the mean % RSD was found to be within acceptance limit i.e. (≤2%). Hence it was concluded that there was no significant difference for the assay, which was tested within the day and between the days. So, we concluded that the proposed method at selected wavelength was found to be precise.

Method Robustness

The influence of small changes in optimized chromatographic conditions such as changes in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & Methanol content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-14, RSD (%) < 2%) the proposed RP-HPLC method was used for the analysis of Celecoxib (API).[29]

Table 14: Result of Method Robustness Test for Celecoxib.

Change in Parameter	% RSD
Flow (0.9 ml/min)	1.06
Flow (1.1 ml/min)	0.69
Wavelength of Detection (298 nm)	0.28
Wavelength of Detection (332 nm)	0.14

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & Methanol content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-15, % RSD < 2%) the developed RP-HPLC method for the analysis of Tramadol HCL (API).

Table 15: Result of Method Robustness Test for Tramadol HCL.

Change in Parameter	% RSD
Flow (0.9 ml/min)	0.03
Flow (1.1 ml/min)	0.08
Wavelength of Detection (298 nm)	0.82
Wavelength of Detection (330 nm)	0.46

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantization (LOD and LOQ) can be determined by the following equations [30]. These equations are based on the signal to noise ratio. These two equations are useful for the determination of LOD and LOQ.[31]

L.O.D. = 3.3 (SD/S).
L.O.Q. = 10 (SD/S)
Where,
SD = Standard deviation Response
S = Slope of the Calibration curve

The slope S and standard deviation response values are obtained from the calibration curve of the analyte (Drug).

Observation: The LOD was found to be 0.607 µg/ml and 1.821 µg/ml and LOQ was found to be 0.451 µg/ml and 1.353 µg/ml for Celecoxib and Tramadol HCL respectively which represents that sensitivity of the method is high.

System Suitability

This includes the type of equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be examined.[32] The following system suitability test parameters were determined. The obtained data are shown in Table-16.

Table-16: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	3.56
2	Asymmetry	T ≤ 2	Celecoxib =0.17 Tramadol HCL =0.61
3	Theoretical plate	N > 2000	Celecoxib =3698 Tramadol HCL= 4926

Determination of Celecoxib and Tramadol HCL in Pharmaceutical Dosage form

Each Tablet Contains: 56/44mg

20 tablets were taken and the I.P. method was followed to measure the average weight. Above weighed tablets were finally powdered and triturated well by using mortar and pestle. A quantity of powder equivalent to 100 mg of drug were calculated and transferred to clean & dry 100ml volumetric flask, and add 70 ml of HPLC grade methanol and solution was sonicated for 15 minutes by using Sonicator. Then after volume was made up to 100 ml with same solvent. Then finally 10ml of the above solution was diluted up to 100ml with HPLC grade methanol or same solvent. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (0.1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.[33]

The solution prepared was injected in three replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution (without drug) was injected into the HPLC system and the peak areas were recorded. The data are shown in Table-17.

ASSAY:

Assay % =

%

Assay=AT/AS×WS/DS×DT/WT×P/100×AW/LC×100

Where:

AT = Peak Area of sample obtained with sample preparation

WS = Weight of working standard taken in mg

AS = Peak Area of standard obtained with standard preparation

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

P = Percentage purity of working standard

DT = Dilution of sample solution

The assay was performed explained in above chapter.[34]

Results obtained are tabulated in below:

Table-17: Assay of Celecoxib & Tramadol HCL in Pharmaceutical Dosage form

Brand Name of Tablets	Labelled Amount of Drug (mg)	Mean (±SD) Amount (mg) found by the Proposed Method (n=6)	Assay + % RSD
Seglentis Tablets (Esteve Pharmaceuticals, S.A.)	56/44	55.468 (± 0.452) /43.582 (± 0.324)	99.427/99.385 (± 0.486)

Observation: The assay of Seglentis Tablets containing CELECOXIB and TRAMADOL HCL was found to be 99.427% and 99.486% respectively.

Stability Studies

Results of Degradation Studies: The results of the forced degradation studies indicated the **Specificity** of the developed method that has been developed. Celecoxib and Tramadol HCL were stable in thermal and photolytic stress conditions.[35] The results of stability studies are given in the following Table-18.

Table-18: Results of Stress Studies of Celecoxib and Tramadol HCL API.

Stress Condition	Time (hours)	Assay of Active Substance	Assay of Degraded Products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	93.05	6.95	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	97.11	2.89	100.00
Thermal Degradation (50 ^{0}C)	24Hrs.	63.22	36.78	100.00
Photolytic Degradation (UV 254nm)	24Hrs.	87.65	12.35	100.00
Oxidation Degradation	24Hrs.	96.44	3.56	100.00

SUMMARY AND CONCLUSION

From the results shown in system suitability the %RSD for retention times, peak areas and number of theoretical plates and tailing factor were found to be within limits i.e., %RSD for retention times not more than 2.0%, peak areas not more than 2.0% and number of theoretical plates not less than 2000 and tailing factor for not more than 2.0, so they had method passed system suitability. From the results shown in precision tables it was found that % RSD is not more than 2%; which indicates that

the proposed method has good reproducibility. In case of accuracy 80%, 100% and 120% of solutions with respect to target assay concentrations the percentage recovery for each levels are between 98.0 %to 102%. It indicates the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering the proposed method. From the results shown in Linearity table it was found that the method was linear and the correlation coefficient is not less than the 0.9999. In case of the LOD and LOQ the S/N ratios are within the limits for Celecoxib / Tramadol HCL.

The proposed method was found to be rapid, accurate, precise, specific, robust and economical. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. This method is also having an advantage than reported method that the retention time of both the drugs is below 4 mins and both the drugs can be assayed with the short time. Thus the method is not time consuming and can be used in laboratories for the routine analysis of combination drugs.

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