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QUANTITATIVE ESTIMATION OF CELECOXIB AND TRAMADOL HYDROCHLORIDE IN SYNTHETIC BINARY MIXTURE BY RP-HPLC

[1]PEDDI VINAY KUMAR, [2]Dr.A.YASODHA, [3]MANTHA VENKATESH

[1]Student, [2]Professor cum Principal, [3]Associate Professor
[1]Pharmaceutical analysis,
[1]Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar, Telangana, India

Abstract : An analytical simple, reproducible and efficient RP-HPLC method was developed for quantification of Celecoxib and Tramadol Hcl in synthetic binary mixture. This Separation was carried out on Targetsil C18(250 x 4.6mm, 5µm particle size) column in isocratic mode with mobile phase containing Methanol: TEA Buffer pH 7.4: Acetonitrile (65:15:20) pH 7.4 . The flow rate was 1.0 ml/min and effluent was monitored at 225 nm. The retention times for Celecoxib and Tramadol Hcl were 2.087 and 5.268 min respectively. The method was validated as per ICH guidelines. The developed method was found to be accurate, precise and selective.

IndexTerms **- Celecoxib, Tramadol Hcl, RP-HPLC, Synthetic binary mixture**

I. INTRODUCTION

Literature survey reveals certain spectrophotometric methods were reported for estimation and there is a method is available for such estimation by RP-HPLC. In view of the need for a suitable RP-HPLC method for routine analysis of Celecoxib and Tramadol Hcl in formulations, attempts were made to develop simple, precise and accurate analytical method for Quantitative estimation of Celecoxib and Tramadol Hcl and extend it for their determination in formulation. Validation is a necessary and important step in both framing and documenting the capabilities of the developed method. The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient.

II. RESEARCH METHODOLOGY

Preparation of standard solution:
Accurately weigh and transfer 10 mg of Celecoxib and Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15ml of the above Celecoxib and 0.1.35ml of Tramadol Hcl stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.
Procedure: Inject the samples by changing the chromatographic conditions and record the Chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.
Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 65:15:20 v/v respectively.
Optimization of Column:
The method was performed with Targetsil C18 (4.6×150mm, 5µ) column which was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Shimadzu LC-10 AT VP
Temperature	:	Ambient
Column	:	X-Terra C18 (4.6×150mm, 5µ)

Mobile phase : Methanol: TEA buffer: ACN (65:15:20v/v)
Flow rate : 1ml/min
Wavelength : 225 nm
Injection volume : 10 µ l
Run time : 10 min

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Celecoxib and 10mg of Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the above Celecoxib and 1.35ml of Tramadol Hcl stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Celecoxib and 10mg of Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the above Celecoxib and 1.35ml of Tramadol Hcl stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Celecoxib and Tramadol Hcl sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1.35ml of Sample stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: Inject the three replicate injections of standard and sample solutions and calculate the Quantity by using formula:

$$\textbf{Amount of drug} = \frac{\text{Peak area of sample}}{\text{Peak area of Standard}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \text{Avg wt}$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Celecoxib and 10mg of Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (5ppm of Celecoxib & 45ppm of Tramadol Hcl):

Pipette out 0.05ml of Celecoxib and 0.45ml of Tramadol Hcl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10 ppm of Celecoxib&90ppm of Tramadol Hcl):

Pipette out 0.1ml of Celecoxib and 0.9ml of Tramadol Hcl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15ppm of Celecoxib&135ppm of Tramadol Hcl):

Pipette out 0.15ml of Celecoxib and 1.35ml of Tramadol Hcl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20 ppm of Celecoxib&180ppm of Tramadol Hcl):

Pipette out 0.2ml of Celecoxib and 1.8ml of Tramadol Hcl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25 ppm of Celecoxib&225ppm of Tramadol Hcl):

Pipette out 0.25ml of Celecoxib and 2.25ml of Tramadol Hcl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

REPEATABILITY

Preparation of Celecoxib and Tramadol Hcl Product Solution for Precision:

Accurately weigh and transfer 10 mg of Celecoxib and 10mg of Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the above Celecoxib and 1.35ml of Tramadol Hcl stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.The standard solution was injected for six times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

DAY 2: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ACCURACY:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Celecoxib and 10mg of Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.075ml of the above Celecoxib and 0.67ml of Tramadol Hcl stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Celecoxib and 10mg of Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the above Celecoxib and 1.35ml of Tramadol Hcl stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Celecoxib and 10mg of Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.22ml of the above Celecoxib and 2.02ml of Tramadol Hcl stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the Chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Celecoxib and Tramadol Hcl and calculate the individual recovery and mean recovery values.

ROBUSTNESS:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Celecoxib and 10mg of Tramadol Hcl working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the above Celecoxib and 1.35ml of Tramadol Hcl stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

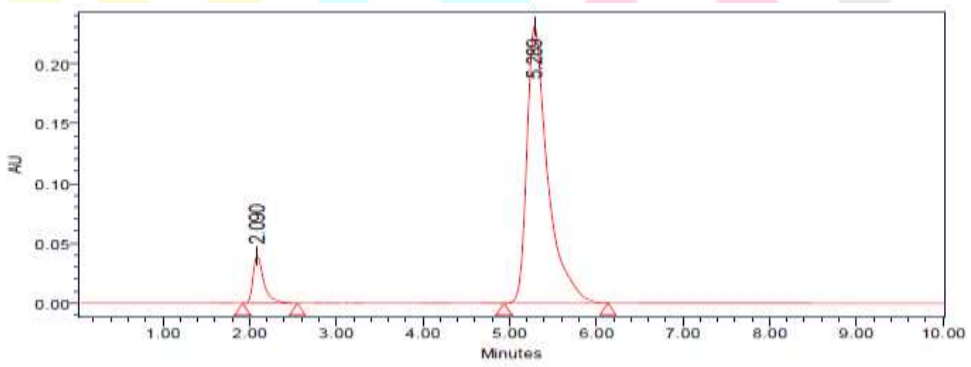
Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and Chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer: Acetonitrile was taken in the ratio and 70:5:25, 60:30:10 instead (65:15:20), remaining conditions are same. 10µl of the above sample was injected and Chromatograms were recorded.

III. RESULTS AND DISCUSSION



Optimized Chromatogram (Standard)

Table: - Peak results for optimized (Standard)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Celecoxib	2.090	372126	39690		1.70	5587
2	Tramadol Hcl	5.289	3864998	231194	9.80	1.77	5698

Table: Results of system suitability for Tramadol Hcl

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Tramadol Hcl	5.289	3864998	231194	5786	1.46	9.80
2	Tramadol Hcl	5.289	3864998	232184	5908	1.47	9.81
3	Tramadol Hcl	5.338	3881443	231044	5487	1.48	9.81
4	Tramadol Hcl	5.327	3896952	231969	5032	1.40	9.83
5	Tramadol Hcl	5.262	3900103	233541	5389	1.43	9.82
Mean			3881699				
Std. Dev			16802.33				
% RSD			0.4				

Table: Peak results for Quantitative estimation of Synthetic mixture

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Celecoxib	2.088	352290	40269		1.69	5516	1
2	Tramadol Hcl	5.276	3883794	231354	9.75	1.89	5677	1
3	Celecoxib	2.087	356547	41157		1.72	5557	2
4	Tramadol Hcl	5.268	3896493	234961	9.82	1.91	5804	2
5	Celecoxib	2.085	358914	40963		1.75	5489	3
6	Tramadol Hcl	5.262	3900103	233541	9.78	1.95	5790	3

Table: The Quantity of Celecoxib and Tramadol Hcl in synthetic mixture was found to be 54.9mg & 43.2mg

	Celecoxib	Tramadol Hcl	Quantity Found
Seglentis Tablet	56 mg	44mg	--
Proposed Synthetic mixture composition	56 mg	44mg	54.9mg/43.2mg

Table: Calibration data for Celecoxib:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	134436
66.6	10	245571
100	15	371548
133.3	20	499024
166.6	25	619830

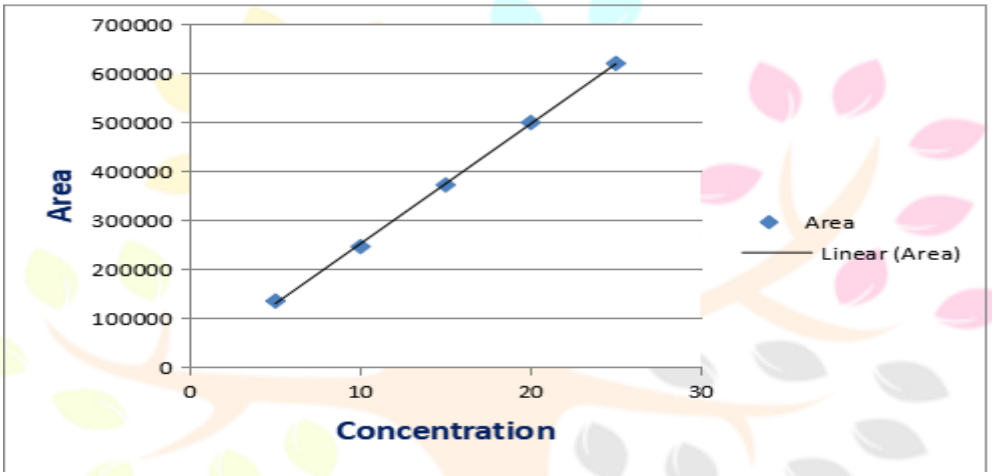


Figure: calibration graph for Celecoxib

Calibration data for Tramadol Hcl

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	45	1330054
66	90	2728974
100	135	3917063
133	180	5300022
166	225	6412695

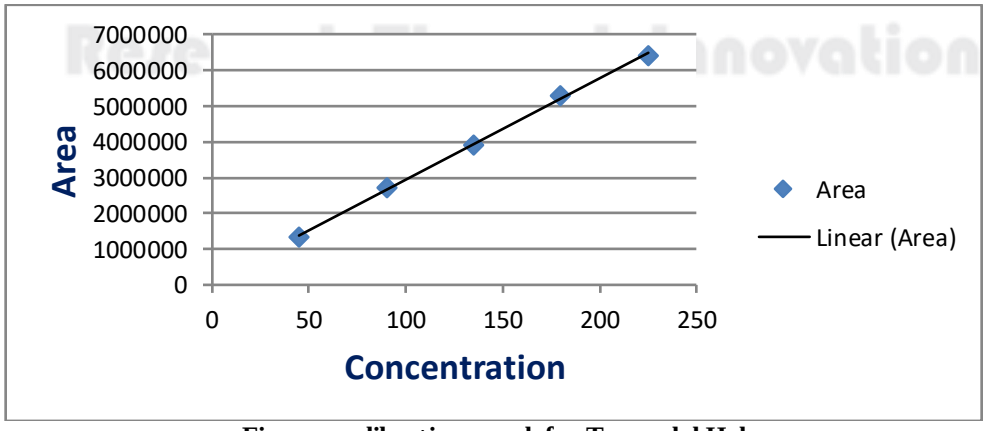


Figure : calibration graph for Tramadol Hcl

Table: Results of repeatability for Celecoxib:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Celecoxib	2.086	362266	41697	5081.3	1.8
2	Celecoxib	2.083	364902	41402	5144.1	1.9
3	Celecoxib	2.083	366870	41540	5118.1	1.8
4	Celecoxib	2.081	367273	42256	5147.3	1.8
5	Celecoxib	2.081	368101	42143	5101.8	1.8
6	Celecoxib	2.085	367185	42963	5134	1.8
Mean			365882.4			
Std. Dev			2338.4			
% RSD			0.6			

Table: Results of repeatability for Tramadol Hcl:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Tramadol Hcl	5.178	3903548	240181	5988.3	2.0	9.8
2	Tramadol Hcl	5.199	3905819	235523	5856.3	2.0	9.7
3	Tramadol Hcl	5.235	3916120	238578	5930.2	2.0	9.9
4	Tramadol Hcl	5.202	3916542	238814	5936.9	2.0	9.8
5	Tramadol Hcl	5.206	3920943	241006	5040.0	2.0	9.5
6	Tramadol Hcl	5.2202	3920943	241467	5048.0	2.0	9.5
Mean			3912594.4				
Std. Dev			7507.6				
% RSD			0.2				

Table: Results of Intermediate precision Day1 for Celecoxib

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Celecoxib	2.083	369246	42277	5537.8	1.6
2	Celecoxib	2.083	370766	42708	5561.8	1.6
3	Celecoxib	2.089	370840	42065	5489.3	1.6
4	Celecoxib	2.083	370840	42065	5489.3	1.6
5	Celecoxib	2.082	371041	42568	5583.2	1.8

6	Celecoxib	2.080	371386	42211	5533.2	1.8
Mean			370686.5			
Std. Dev			740.7369			
% RSD			0.19			

Table: Results of Intermediate precision Day 1 for Tramadol Hcl

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Tramadol Hcl	5.229	3743003	242955	5269.7	2.2	10.2
2	Tramadol Hcl	5.203	3845359	242255	5100.5	2.1	10.0
3	Tramadol Hcl	5.133	3885014	242854	5127.6	2.1	10.0
4	Tramadol Hcl	5.229	3743003	242955	5269.7	2.2	10.2
5	Tramadol Hcl	5.151	3722513	240346	5048.8	1.5	9.9
6	Tramadol Hcl	5.112	3728789	237638	5997.2	1.6	9.9
Mean			3777947				
Std. Dev			69194.4				
% RSD			1.8				

Table: Results of Intermediate precision Day 2 for Celecoxib

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Celecoxib	2.078	370979	42978	7083.0	1.9
2	Celecoxib	2.082	371041	42568	8583.2	1.8
3	Celecoxib	2.080	371386	42211	7533.2	1.8
4	Celecoxib	2.089	369246	42277	6537.8	1.6
5	Celecoxib	2.083	370840	42065	5489.3	1.6
6	Celecoxib	2.089	369246	42277	6537.8	1.6
Mean			370456.3			
Std. Dev			954.6004			
% RSD			0.25			

Table: Results of Intermediate precision for Tramadol Hcl

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Tramadol Hcl	5.077	3841404	246818	5208.0	1.5	10.1
2	Tramadol Hcl	5.151	3885014	242854	5127.6	1.3	10.0
3	Tramadol Hcl	5.112	3743003	242955	5269.7	1.5	10.2
4	Tramadol Hcl	5.133	3743003	242955	5269.7	1.6	10.2
5	Tramadol Hcl	5.203	3885014	242854	5127.6	1.5	10.0
6	Tramadol Hcl	5.133	3743003	242955	5269.7	1.6	10.2
Mean			3806740				
Std. Dev			71613.47				
% RSD			1.8				

The accuracy results for Celecoxib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	192446.6	7.5	7.4	98.6	98.7%
100%	374222	15	14.8	98.66	
150%	555891.3	22.5	22.3	99.1	

The accuracy results for Tramadol Hcl

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2001752	67.5	67.3	99.7	99.7%
100%	3927797	135	134.8	99.8	
150%	5858665	202.5	202.1	99.8	

LIMIT OF DETECTION

Celecoxib: 0.6µg/ml Tramadol Hcl: 9.7µg/ml

LIMIT OF QUANTITATION

Celecoxib: 2.0µg/ml Tramadol Hcl: 29.4µg/ml

ROBUSTNESS

Celecoxib:

Parameter used for synthetic mixture analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	372126	2.090	5587	1.70
Less Flow rate of 0.9 mL/min	356765	2.736	5432	1.82
More Flow rate of 1.1 mL/min	342356	1.673	5644	1.91
Less organic phase	312434	2.736	5098	1.82
More organic phase	305623	1.673	5123	1.91

Tramadol Hcl:

Parameter used for synthetic mixture analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3864998	5.289	5698	1.77
Less Flow rate of 0.9 mL/min	3546737	6.746	5546	1.88
More Flow rate of 1.1 mL/min	3857216	4.032	5124	1.91
Less organic phase	3810347	6.746	5034	1.88
More organic phase	3875642	4.032	5612	1.91

IV CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Celecoxib and Tramadol Hcl in bulk drug and pharmaceutical dosage forms.This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.Celecoxib and Tramadol Hcl was freely soluble in ethanol, methanol and sparingly soluble in water.Methanol: TEA Buffer pH 7.4: Acetonitrile (65:15:20) was chosen as the mobile phase. The solvent system used in this method was economical. The analytical method was found linearity over the range 5-25mg/ml of Celecoxib and 45-225 mg/ml of Tramadol Hcl of the target concentration.
The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.This method can be used for the routine determination of Celecoxib and Tramadol Hcl in bulk drug and in Pharmaceutical dosage forms.

VI ACKNOWLEDGMENT

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REFERENCES

[1] Katravath Sony,M. Venkatesh and A.Yasodha,RP-HPLC Method Development And Validation of Celecoxib And Tramadol Hydrochloride, World Journal of Pharmaceutical and Life Science,2024,10(9),28-140.

[2] P Kunjan, P Bhumi, P Ankita, P Divyakant, P Jaymin, Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Celecoxib and Tramadol HCL in Synthetic Mixture, International Journal of Creative Research Thoughts(IJCRT),2023,11(3),307-320.

[3].Hesham Sameh Ramadan, Randa A. Abdel Salam, Ghada M. Hadad, Fathalla Belal, and Mohamed M. Salim , Eco-friendly simultaneous multi-spectrophotometric estimation of the newly approved drug, 2023,13(1) ,11716-11728