ANALYTICAL METHOD VALIDATION/VERIFICATION REPORT FOR ASSAY OF CELECOXIB CAPSULES

1. Summary

High Performance Liquid Chromatographic method for the determination of Assay in Celecoxib Capsules has been carried out using High Performance Liquid chromatography (HPLC) system. High Performance Liquid chromatographic method for the determination of Assay has been validated. The method is found to be linear, precise, specific, accurate, rugged and robust for the intended studies and therefore suitable for use in determining the assay in Celecoxib Capsules 400 mg.

Table of content

The method is studied for following parameters,

Content	Observation		Acceptance Criteria
Specificity	There is no any interference of Placebo of Celecoxib Capsules 400 mg and blank with the Celecoxib peak. Theoretical plate count For Celecoxib Peak is : 10896 Tailing factor for Celecoxib Peak : 1.33 Peak purity is Passed by PDA.		Any other placebo peaks should not interfere with Celecoxib peak. Theoretical plate count is: Not less than -2000 Tailing factor: Not more than -2.0 Peak purity should be Passed by DA.
Precision			
System Precision	**Standard**	**%RSD**	Relative standard deviation (%RSD) for peak area of Celecoxib with six replicate injections of standard preparation is less than 2.0%
	Celecoxib	0.23	

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Content	Observation		Acceptance Criteria
Method Precision	**Result (mg)**	**%Assay**	- Relative Standard deviation (%RSD) for Assay of six different sample preparations: Not more than - 2.0%
	400.09	100.02	
	397.88	99.47	
	400.63	100.16	
	399.47	99.87	
	400.85	100.21	
	402.10	100.52	
Mean	**400.17**	**100.04**	
% RSD	**0.36**	**0.36**	
Intermediate Precision (Ruggedness)			
System Precision	**Standard**	**%RSD**	Relative standard deviation (%RSD) for peak area of Celecoxib with six replicate injections of standard preparation is less than 2.0%
	Celecoxib	0.16	
Method Precision	**Result (mg)**	**%Assay**	
	405.42	101.36	
	400.11	100.03	
	402.85	100.71	
	403.24	100.81	
	405.51	101.38	
	407.19	101.80	
Mean	**404.05**	**101.01**	
% RSD	**0.62**	**0.62**	
Summary for overall 12 Assay	Overall Relative Standard deviation (%RSD) of assay for Celecoxib Capsules with 12 determinations is 0.70%		Relative Standard deviation (%RSD) for Assay of twelve different sample preparations: Not more than - 2.0%
Accuracy as Recovery	Recovery for assay from the sample obtained with triplicate test preparation at each level (I.e. about 50%, 100%, 150 % of specification level) is in the limit of 98-102%.		Recovery for assay from the sample obtained with triplicate test preparation at each level (i.e. about 50%, 100%, 150 % of specification level) should be between 98 and 102 %
Prep. No	**50%**	**100%**	**150%**
1	99.66	99.52	100.04
2	100.09	100.67	100.57
3	99.62	100.08	99.61

Linearity	**Active Ingredient**	**Correlation Coefficient (R^2)**	- The Correlation Coefficient (R^2) should be not less than 0.99
	Celecoxib	0.99	
Robustness	The system suitability conditions are met at condition (column flow changed to 0.9 ml/ minute & 1.1 ml/minute		- System suitability should pass after making small changes in the method parameters.

"The analytical data of each study shows satisfactory results against acceptance criteria defined in the Protocol, hence it is concluded that method is validated for above parameters and suitable its intended use for the determination of assay in Celecoxib Capsules 40

2. Introduction:

This report describes the validation of test procedure used for the determination of assay in Celecoxib Capsules. The analytical methodology used for HPLC system. The method was validated as per validation protocol.

3. Objective:

The objective of this analytical method is to demonstrate that it is suitable for its intended purpose. The overall purpose of the validation is to provide documented evidence of specificity, precision and accuracy, linearity for the method with the help of the following parameters.

- Specificity & Peak purity
- Precision
  - System Precision
  - Method Precision
  - Intermediate precision (Ruggedness)
- Accuracy as recovery
- Linearity & Range
- Robustness

Detail of method, each experiment, and observations during the performance and results are reported below.

4. Analytical Methodology:

Chromatographic Conditions:

Column	: C_{18}, (5µm, 250mm x 4.6 mm)
Flow Rate	: 1.0 ml/min
Wave length	: 238 nm
Injection Volume	: 20 µl
Column oven Temperature	: Ambient

Mobile phase:

10 volumes Acetonitrile, 10 volumes Methanol and 5 volumes of water. Filter and degassed.

Diluent preparation: mobile phase

5. Working standard and sample used

Working standard

Name	:	Celecoxib
Working std Id.	:	.....
Purity	:	98.86 %

6. SPECIFICITY:

Separately injected blank, placebo solution, spiked solution (Duplicate injections). In the Blank, placebo injection showed no significant peaks at the Retention time of Celecoxib in the chromatograms

Conclusion: There is no interference from blank and placebo and Peak purity is passed by PDA. Hence the method is found to be specific

7. PRECISION:

7.1 Precision

7.1.1 System Precision

System precision was performed by injecting six replicate injections of standard solution at 100% specification level and results are shown in the Table – 2.

Table –2: System Precision

Celecoxib		
Injection No.	**Retention time**	**Area**
1	5.605	10937355
2	5.606	10932808
3	5.597	10963543
4	5.597	10949016
5	5.597	10960115
6	5.594	11001241
Mean	**5.599**	**10957346.33**
Std. Dev.	**0.005**	**24673.81**
RSD (%)	**0.09**	**0.23**

Conclusion: Relative standard deviation (%RSD) for Celecoxib peak area with replicate injections is less than 2.0%.

7.1.2 Method precision:

Precision of the method was demonstrated by calculating the assay with six different sample preparations. Results found to be within the acceptance limit and RSD is 0.36% Results are shown in table-3

Table –3: Method precision

Sample Preparation	Result (mg)	%Assay
1	400.09	100.02
2	397.88	99.47
3	400.63	100.16
4	399.47	99.87
5	400.85	100.21
6	402.10	100.52
Mean	**400.17**	**100.04**
RSD (%)	**0.36**	**0.36**

Conclusion: The method is found to be precise.

7.2 Intermediate precision (Ruggedness):

This study was carried out as per method precision by a different analyst on a different day with different instrument by using different set of standard solution and sample solution.

7.2.1 System Precision:

System precision was performed by injecting six replicate injections of standard solution at 100 % specification level and results are shown in the Table – 4.

Table –4: System Precision

Celecoxib		
Inj. No.	**Retention time**	**Area**
1	5.677	11489689
2	5.702	11498118
3	5.718	11520926
4	5.729	11521643
5	5.732	11532886
6	5.738	11531932
Mean	**5.716**	**11515866**
Std. Dev.	**0.023**	**17927.64**
RSD (%)	**0.40**	**0.16**

Conclusion: Relative standard deviation (%RSD) for Celecoxib peak area with replicate injections is with in the limit.

7.2.2 Method precision:

Intermediate Precision of the method was demonstrated by calculating the assay with six different sample preparations. Results found is 0.62% of 6 assays RSD results are shown in the Table – 5

Table –5: Method precision

Sample Preparation	Result (mg)	%Assay
1	405.42	101.36
2	400.11	100.03
3	402.85	100.71
4	403.24	100.81
5	405.51	101.38
6	407.19	101.80
Mean	**404.05**	**101.01**
RSD (%)	**0.62**	**0.62**

% RSD of assay for 12 sample preparations between different analysts

Table 6:

Sample Preparation	Celecoxib Assay (%)
1	100.02
2	99.47
3	100.16
4	99.87
5	100.21
6	100.52
7	100.02
8	99.47
9	100.16
10	99.87
11	100.21
12	100.52
Mean	**100.53**
RSD (%)	**0.70**

CONCLUSION:

The % RSD for the overall assay of the twelve sample preparations is with in the limits

8. Accuracy as recovery

The accuracy of the method was demonstrated at three different concentration levels by calculating recovery (about 50 %, 100 %, and 150 % of specification level). The method is found to be accurate. Results are shown in table-7

Table –7: % Recovery

% Level	Sample Preparation	Celecoxib %Recovery
50 %	1	99.66
	2	100.09
	3	99.62
100 %	1	99.52
	2	100.67
	3	100.08
150 %	1	100.04
	2	100.57
	3	99.61

CONCLUSION:

% Recovery complies with specified acceptance criteria, hence the method is found to be accurate in the range of 50 to 150%.

9. Linearity and Range

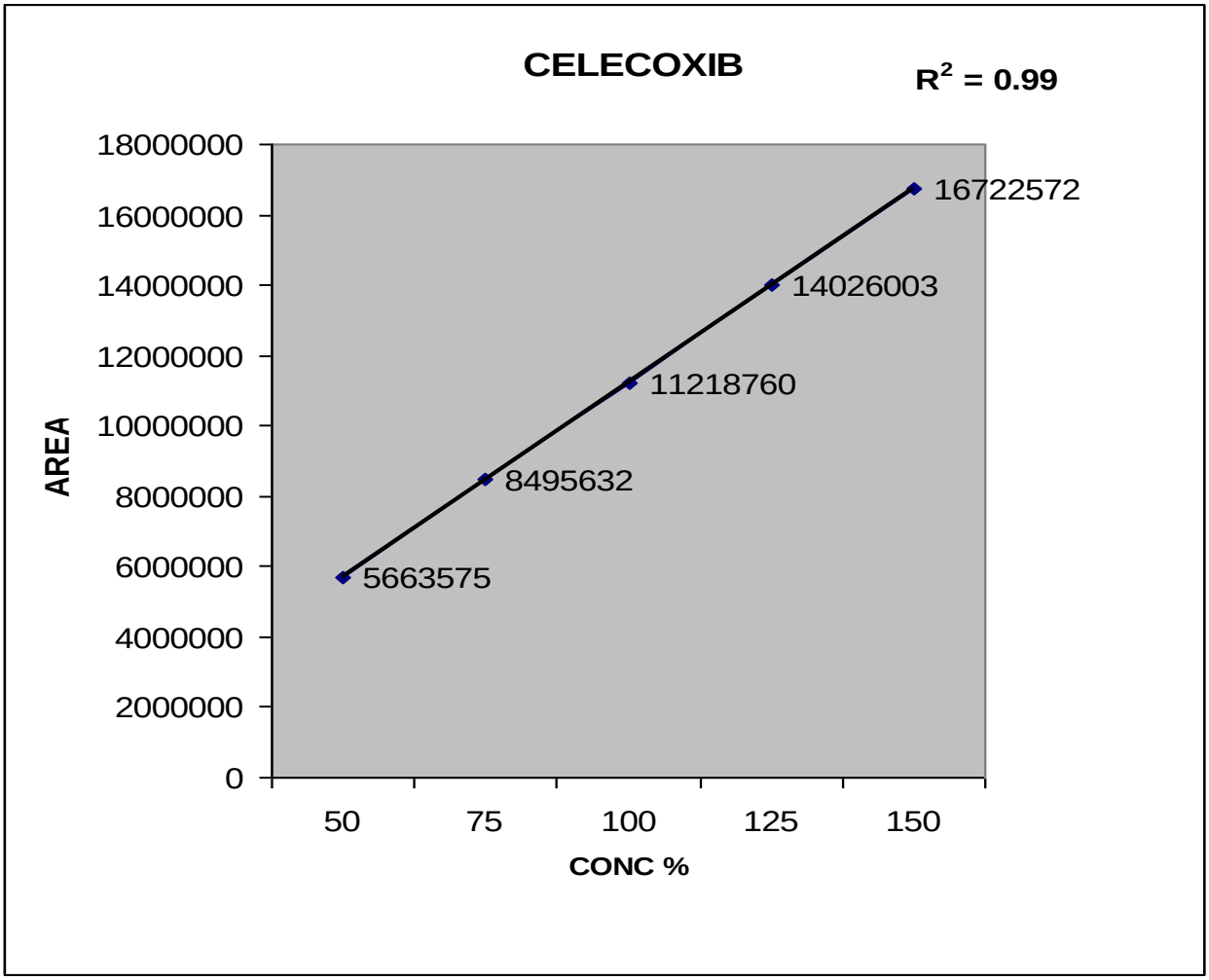
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

- Established the linearity over a range of five different concentrations of the analyte from 50 %, 75%, 100%, 125% and 150% of specification level, injected each concentration in triplicate.

- Plot a graph with concentration against response (area count) and calculate the correlation coefficient. Results are shown in table-8

Table –8: Linearity for Celecoxib

S.No.	%	Concentration (ppm)	Mean Area
1.	50.0	100.0	5663575
2.	75.0	150.0	8495632
3.	100.0	200.0	11218760
4.	125.0	250.0	14026003
5.	150.0	300.0	16722572
Correlation coefficient (R^2)			**0.99**



Conclusion: The Correlation Coefficient (R^2) is 0.99 for Celecoxib. Hence method is linear in the range of 50 to 150% of specification level.

10. Robustness:

The robustness of an analytical method 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.

Robustness was demonstrated by changing flow rate to 0.9 ml/min & 1.1 ml/min inject six injections of Standard preparation as per methodology.

10.1 Flow Rate at 0.9 ml / minute

Table –9: Observation of system suitability parameters

Inj. No.	Retention Time	Area	Theoretical Plates	Tailing Factor
1	6.187	12463195	10316	1.30
2	6.183	12452269	10312	1.30
3	6.176	12543729	10325	1.30
4	6.177	12667681	10208	1.30
5	6.170	12569243	10368	1.30
6	6.181	12459219	10333	1.30
Mean	**6.179**	**12525889**	**10310**	**1.30**
STD.DEV.	**0.006**	**84968**	**54**	**0.00**
RSD (%)	**0.10**	**0.68**	**0.52**	**0.00**

10.2 Flow Rate at 1.1 ml / minute

Table –10: Observation of system suitability parameters

Inj. No.	Retention Time	Area	Theoretical Plates	Tailing Factor
1	5.155	10724645	11282	1.20
2	5.155	10636350	11265	1.20
3	5.161	10574894	11263	1.20
4	5.158	10614916	11228	1.20
5	5.160	10561187	11245	1.20
6	5.158	10649270	11254	1.20
Mean	**5.158**	**10626877**	**11256**	**1.20**
STD.DEV.	**0.002**	**58839**	**19**	**0.00**
RSD (%)	**0.05**	**0.55**	**0.16**	**0.00**

CONCLUSION:

System suitability parameters meets pre defined acceptance criteria while making changing flow rate.

Conclusion of overall Study for Celecoxib in Celecoxib Capsules 400 mg Analytical Method Validation

The assay by HPLC method adopted for Celecoxib in Celecoxib Capsules is validated, found to be precise, linear and accurate; it is also proved to be rugged & robust, so this method can be used for routine analysis and stability studies.

Prepared By (Sign/Date)	**Reviewed By (Sign/Date)**	**Approved By (Sign/Date)**