Research Article

Stability indicating HPLC method for celecoxib related substances in solid dosage forms

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ABSTRACT

The main objective of the research work was to develop a simple, accurate, stability indicating RP-HPLC method for the quantification of celecoxib and its related substances which can be able to quantify the degradation products and also to get good baseline separation between celecoxib and its process related impurities and degradation products. The method was developed by Agilent HPLC with the column L11, (4.6x250mm, 5μ), Supelcosil DP, it has a mobile phase of Mixture of Buffer, Methanol and Acetonitrile in the ratio of 60: 30: 10v/v/v was used. The flow rate was set at 1.3 ml/min with a detection wavelength of 215nm using VWD detector. The method was validated for analytical parameters such as specificity, accuracy, precision, robustness and ruggedness as per ICH guidelines. The linearity was found to be in the range of 25-120 μg/ml with a correlation coefficient value 0.9991, 0.9986, 0.9990, 0.9992 and 0.9990.Hence this method can be used for routine analysis.

***Key words:*** celecoxib; HPLC; Stability indicating; method development; validation

INTRODUCTION

Celecoxibis chemically known as 4-[5-(4-Methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzenesulfonamide. Its chemical formula and molar mass were $C_{17}H_{14}F_3N_3O_2S$ and 381.373 g/mol. It is a COX-2 selective non-steroidal anti-inflammatory drug (NSAID).It is used to treat the pain and inflammation of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute pain in adults, painful menstruation, and juvenile rheumatoid arthritis[1].Celecoxib is also used for the treatment of colon cancer, ultraviolet light induced skin cancer and breast cancer[2].

The development and validation of an analytical method is to ensure a specific, accurate and precise method for a particular analyte. The principal objective for that is to enhance the conditions and parameters, which should be observed in the evolution and establishment. Literature review reveals that a few analytical methods are developed for the determination of celecoxib using XRD[3], LC-MS[4] and HPLC in bulk and capsules. So far there is no method for stability indicating assay method for celecoxib using HPLC. Hence the author developed a new simple, accurate and stability indicating HPLC method for the determination of celecoxib drug. The method developed was validated as per ICH guidelines[5-12]. The structure of celecoxib and its impurities are shown in the fig.1.
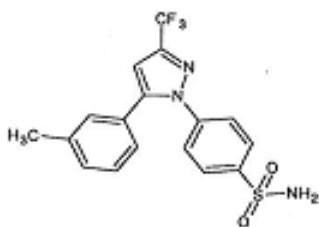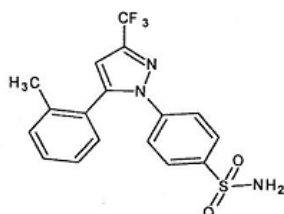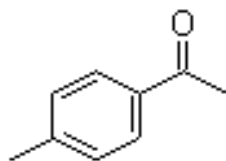
Fig 1: Structure of celecoxib and its impurities
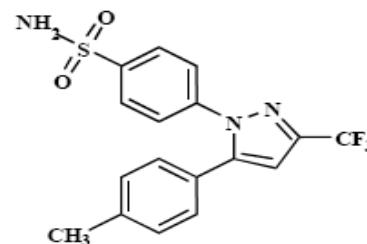
Celecoxib
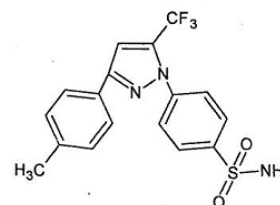
Impurity A (Regio Isomer)

Impurity C (Meta Isomer)

Impurity D (Ortho Isomer)

4-methylacetophenone

MATERIALS AND METHODS

Chemicals and reagents

The Samples of Celecoxib and its impurities were obtained from Fortunee Laboratories (P) Ltd, Kakinada, and Andhra Pradesh, India. All other analytical reagents such as Ammonium formate, acetonitrile, hydrochloric acid, sodium hydroxide and hydrogen peroxide (30%) were obtained from Merck specialty chemicals, Mumbai, India. Milli 'Q' water is used for the preparation of Solutions.

Instrumentation

This research was performed on Agilent make HPLC 1100 instrument. It has binary gradient pump, photo diode array detector (UV), column oven with range of 25°C to 60°C with auto injector. The modules are G1310A isocratic pump with solvent cabinet; G1314A variable wavelength detector (VWD) with standard flow cell (10 mm path length, 14 µl volume, 40 bar maximum pressure) and G2220AA 2D-Value Solution Chem Station.

Chromatographic conditions

The method was developed by Agilent HPLC with the column L11, (4.6x250mm, 5µ), Supelcosil DP, it has a mobile phase of Mixture of Buffer, Methanol and Acetonitrile in the ratio of 60:30:10v/v/v was used. The flow rate was set at 1.3 ml/min with a detection wavelength of 215nm using VWD detector.

The column oven temperature was maintained at 60°C. The injection volume was 25 µl.

Buffer preparation

2.7gm of mono basic potassium phosphate was dissolved in 1000mL of HPLC grade water. pH was adjusted to 3.0 with 10% phosphoric acid.

Mobile phase preparation

Mixture of Buffer, Methanol and Acetonitrile in the ratio of 60:30:10v/v/v was used. Mobile phase was filtered through 0.45µM membrane filter.

Diluent preparation

Diluent buffer was prepared by adding 2ml of TEA and 2ml of phosphoric acid in 1000ml of HPLC water. Mixture of Diluent buffer and acetonitrile in the ratio of 45:55v/v was used as a diluent.

Impurity stock solution preparation

An accurately weighed amount of about 2.4mg of celecoxib related compound A and related compound B were transferred into a 20ml volumetric flask individually. 5ml of diluent was added and sonicated to dissolve. Finally, the volume was made up to the mark with diluent.

System suitability solution preparation

Accurately weighed amount of about 25mg of celecoxib working standard or reference standard was transferred into a 50ml volumetric flask. 10ml of diluent was added and sonicated to dissolve. 1ml of above impurity stock solution was added and then diluted to volume with diluent.

Standard stock solution preparation

An accurately weighed amount of about 25mg of celecoxib working standard or reference standard was transferred into a 50ml volumetric flask. 10ml of diluent was added and sonicated to dissolve. Finally the volume was made up to the mark with diluent. 5ml of above standard stock solution was pipetted out and transferred into a 100ml volumetric flask. Make to the mark with diluent.

Standard solution

4ml of above standard stock solution was pipetted out and transferred into 50ml volumetric flask and then made up to the mark with diluent. The sonication bath temperature was maintained at 20 to 25°C while sonication.

Sample preparation

50 mg of Celecoxib capsule powder was weighed and transferred into 100 mL volumetric flask.

50mL of diluent was added and sonicate for about 10 minutes. Finally the volume was made up to the mark with diluent.

Procedure

25µL portion of diluent as blank, system suitability solution, standard solution (3 times) and test solution was injected into the chromatographic system. The chromatograms were recorded and given in the fig. 2.

Figure 2. Test Chromatogram and Test spiked with impurities chromatogram.
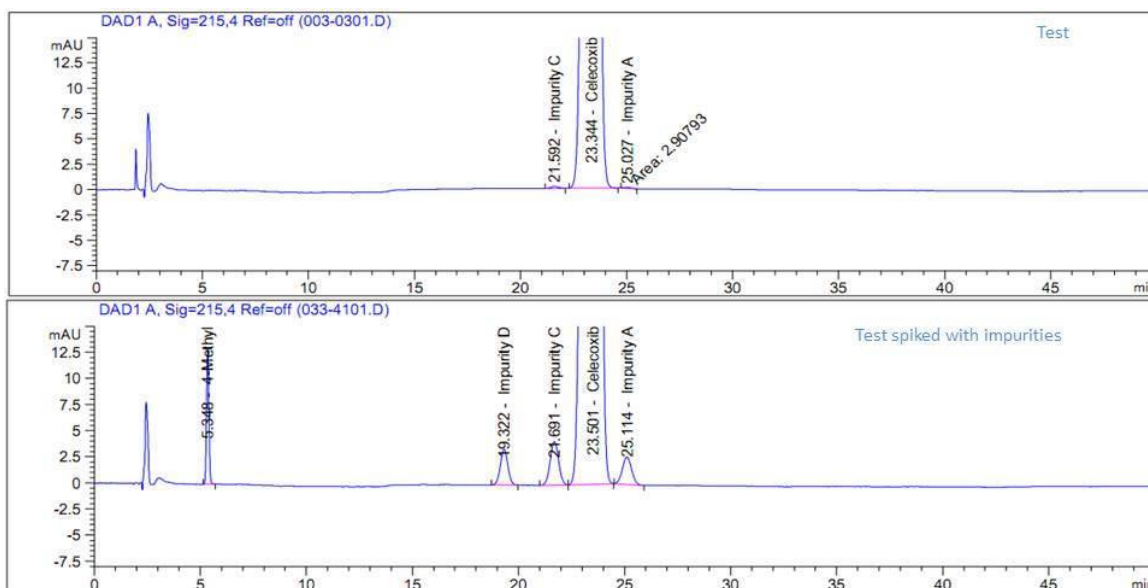
METHOD VALIDATION

The proposed method was validated for the analysis of celecoxib using following parameters. System-suitability studies are an intact part of method development and are practiced to ensure satisfactory performance of the chromatographic system. For five replicate injections of the drugs Number of theoretical plates (N) and tailing factor (T) were assessed. Linearity was established by plotting a graph between concentration versus peak area and the correlation coefficient was determined. To obtain proportionality, the slope and intercept of the regression line and correlation coefficient were calculated statistically from the calibration curve of the celecoxib. To find out variations in the test methods precision was studied for celecoxib of spiked test preparation with celecoxib blend solution to get 0.5% of each impurity with respect to test concentration and analyzed as per test method when analysis carried out by Analyst to Analyst, System to System and Column to Column Variation (ruggedness). The mentioned solution was injected six times and the area was measured for all six injections in HPLC. The % relative standard deviation (%RSD) and % content results were used for assessment of precision and ruggedness. The accuracy of the method was demonstrated by analyzing celecoxib of spiked test preparation with LOQ, 100% and 200% of target concentration. After injection, recovery values for individual drugs were estimated. Specificity is the ability of a method to differentiate the analyte(s) of interest from other components in the sample. Placebo was prepared as per the marketed product formulas of drugs. Placebo interference from excipients was studied. Robustness of the method was determined by varying flow rate, and filtration. Bench top stability (25^0C & 60 % RH) and Refrigerator (8^0C & 55%RH) stability were determined on the 1st and 2nd day. Forced degradation study was conducted to demonstrate the effective separation of degradants from celecoxib. Celecoxib was exposed to the following stress conditions such as refluxed with 3N HCl solution for about 24 hours at 60ºC (Acid). Refluxed with 3N NaOH solution for about 24 hours at 60ºC (Base). Treated with 10% Hydrogen peroxide (H_2O_2) for 24 hours at 60ºC (Peroxide). Dry heat at 105° C for about 24 hrs in an oven.

RESULTS AND DISCUSSION

An Isocratic reverse – phase HPLC procedure was suggested as a suitable method for the analysis of Celecoxib related substances. From the results of optimized method, 5.348, 19.322, 21.691, 23.501 and 25.114 minutes were the retention times for 4-methylacetophenone, Impurity D, Impurity C, Celecoxib and Impurity A respectively. System suitability parameters like theoretical plate, % relative standard deviation and tailing factor for the 4-methylacetophenone, Impurity D, Impurity C, Celecoxib and Impurity A were reported. Mean relative retention factor values of 4-methylacetophenone, Impurity A, Impurity C and Impurity D were found to be 1.09, 0.77, 1.05 and 0.88 respectively.

System suitability parameters

Studies were performed and reported in the table-1.

Table-1. System suitability results

System suitability parameters		Observed value	Acceptance criteria
Theoretical Plates	Celecoxib	15258	Should be NLT 2000
	4-methyl acetophenone	6781	
	Impurity A	5789	
	Impurity C	5687	
	Impurity D	5127	
%RSD	Celecoxib	1.91	Should be NMT 5.0
	4-methyl acetophenone	1.72	
	Impurity A	1.75	
	Impurity C	1.65	
	Impurity D	1.22	
Tailing factor	Celecoxib	1.0	Should be NMT 2.0
	4-methyl acetophenone	1.1	
	Impurity A	1.1	
	Impurity C	1.0	
	Impurity D	1.0	

Forced degradation studies

Forced degradation studies reports shown little deviation in Celecoxib. Purity factor Celecoxib by forced degradation studies was mentioned in table 2. Purity factor of celecoxib was found within the threshold level in all forced degradation studies. Main peak was separated from known impurity and unknown impurities in forced degradation. Mass balance values were within the acceptance limit. (NLT 95.0). The peak purity of Celecoxib was passed in all degradation samples. Celecoxib was very stable in acid, base, oxidation, and thermal condition. Figure 4.6 to 4.13 shows the chromatograms of degradation studies, drug spectra and peak purity factor graph.
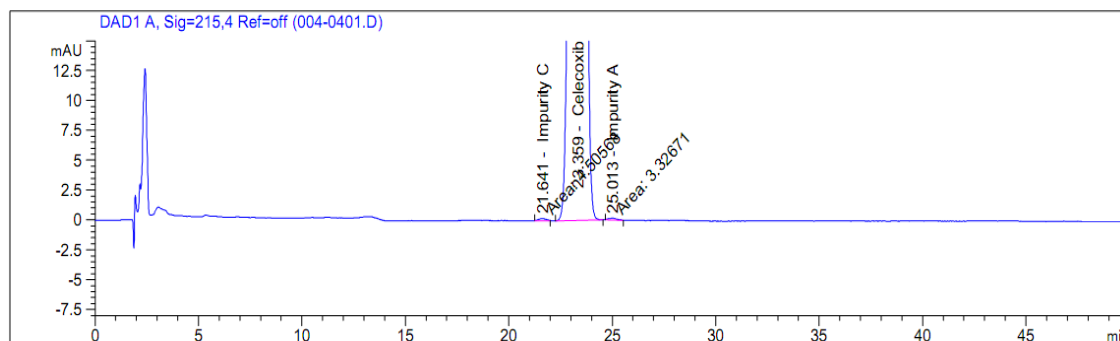
Figure 3. Forced Degradation-Acid Stress

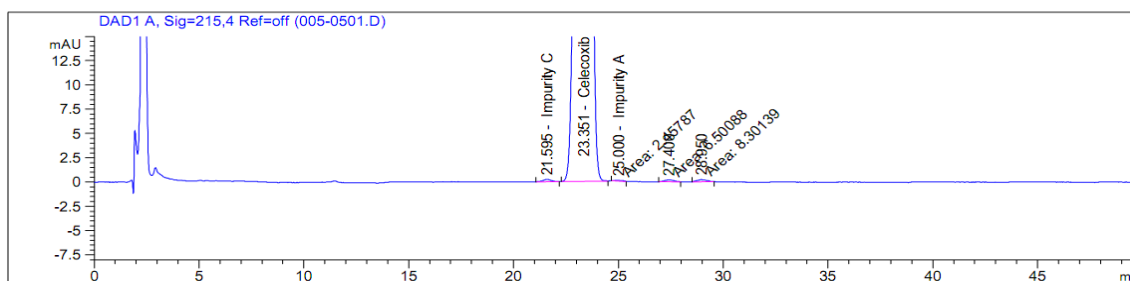**Figure 4. Forced Degradation-Base Stress**
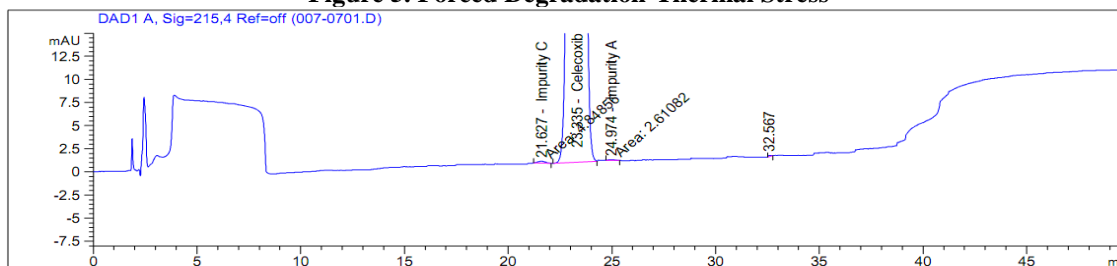
Figure 5. Forced Degradation-Thermal Stress

Figure 6. Forced Degradation-Peroxide Stress

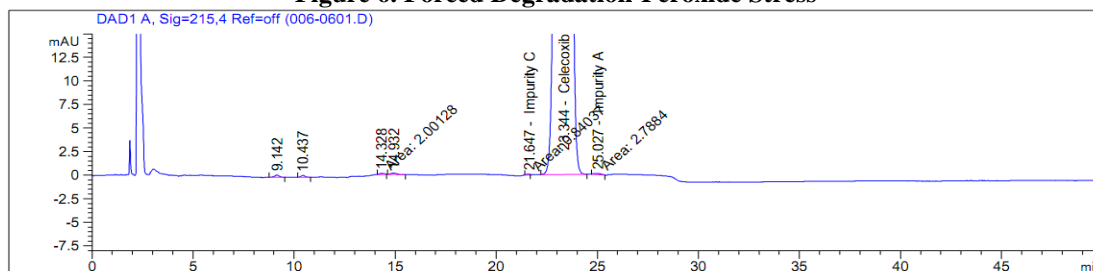**Table 2. Forced degradation studies**

Celecoxib	Purity factor	Threshold limit	Criteria
Unstressed	999.984	990.000	Accepted
Acid stressed	999.983	990.000	Accepted
Base stressed	999.982	990.000	Accepted
Thermal stressed	999.982	990.000	Accepted
H_2O_2 stressed	999.980	990.000	Accepted
Humidity stressed	999.881	990.000	Accepted
UV stressed	999.901	990.000	Accepted
Under sunlight	999.907	990.000	Accepted
By Hydrolysis	999.904	990.000	Accepted

LOQ

Results of LOQ were reported in the table 3. The LOQ values for the impurities were below reporting threshold (0.05%). The test concentration was optimized as 500 PPM.

Linearity

Different concentration of Celecoxib and impurities were analysed. A graph was plotted between concentration and peak area. Correlation coefficient of drugs and its impurities were above 0.99. The Linearity results were summarized in the table 4.8& 4.9. The linearity graphs were shown in figure 4.14. Overlap chromatograms were shown in figure 4.15.

Table 3. LOQ results

Impurity name	% LOQ
4-methylacetophenone	0.0041
Impurity-A	0.0111
Impurity-C	0.0083
Impurity-D	0.0111

Accuracy (% recovery)

A study of accuracy of Celecoxib impurities from spiked samples of test preparation was conducted. Samples were prepared in triplicate at each level by spiking test preparation. The mean % recovery of Celecoxib impurities at mentioned concentration level were reported in the table 4.10. The celecoxib known impurities recovery is should be within the acceptance limit between 85.0% to 115.0%.

Table 4. Linearity data for Celecoxib, Impurity A and C.

Celecoxib		Impurity A		Impurity C	
Mean Conc. (µg/mL)	Mean ± SD	Mean Conc. (µg/mL)	Mean ± SD	Mean Conc. (µg/mL)	Mean ± SD
0.009	3335 ± 9	0.01	3106 ± 7	0.0145	3910 ± 7
0.0253	7237 ± 10	0.0253	35566 ± 87	0.024	4696 ± 8
0.0512	15972 ± 33	0.0511	70836 ± 98	0.049	9806 ± 8
0.083	23792 ± 42	0.081	111882 ± 100	0.079	15240 ± 10
0.1	29223 ± 67	0.1	140434 ± 102	0.099	18939 ± 14
0.12	35177 ± 56	0.12	168862 ± 112	0.122	23603 ± 13
Slope	287036		1468438		185702.5
Intercept	537.0865		-6364.45		705.7122
Correlation coefficient	0.999188		0.998602		0.999077
Conc. – Concentration; SD – standard deviation					

Table 5. Linearity data for Impurity D and 4-methyl acetophenone.

Impurity D		4-methyl acetophenone.	
Mean Concentration (μg/mL)	**Mean ± SD**	**Mean Concentration (μg/mL)**	**Mean ± SD**
0.015	3990 ± 9	0.0156	3840 ± 9
0.025	4890 ± 9	0.024	4910 ± 9
0.051	10006 ± 12	0.049	9945 ± 11
0.081	15640 ± 15	0.081	15046 ± 12
0.098	19039 ± 35	0.099	18965 ± 34
0.129	24603 ± 41	0.128	24610 ± 21
Slope	185188.7695		184676.8467
Intercept	712.946		678.860
Correlation coefficient	0.9992		0.9990

Figure 7. Linearity curve for celecoxib and its impurities.
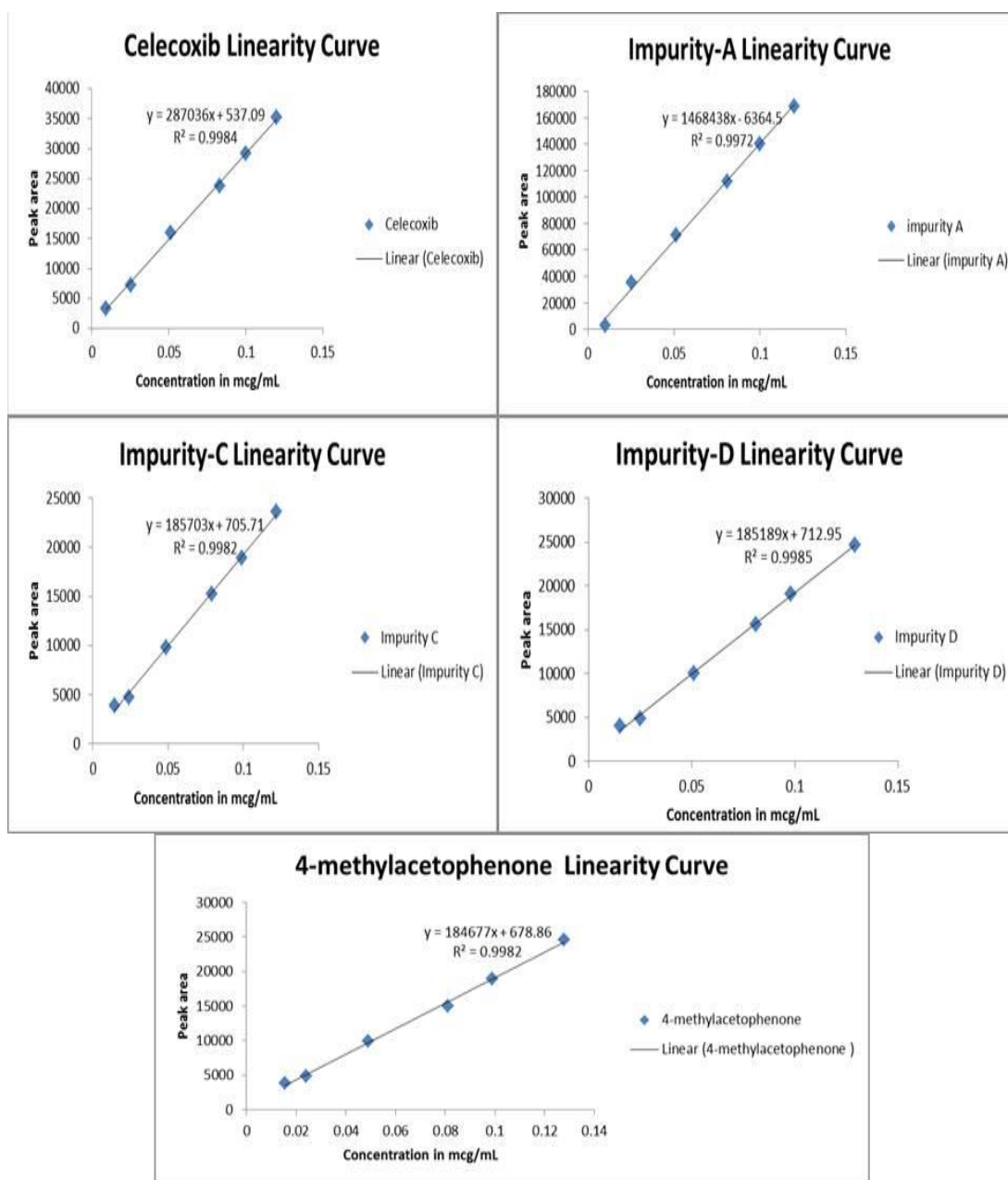
Table 6. Recovery data for Celecoxib impurities.

S. No.	Sample Name	Mean % Recovery			
		Impurity-A	Impurity-C	Impurity-D	4-methyl acetophenone
1	Unspiked	-	-	-	-
2	100% spiked sample-1	96.4	102.1	104.9	105.9
3	100% spiked sample-2	100.3	104.1	108.5	108.2
4	200% spiked sample-1	102.6	105.4	107.9	107.2
5	200% spiked sample-2	101.0	105.6	107.8	107.2

Table 7. System precision results

Injection N°	Response				
	Celecoxib	4-methyl acetophenone	Impurity-A	Impurity-C	Impurity-D
01	1657882.00	4028.65	3531.29	3813.13	4310.49
02	1708883.00	4230.15	3616.83	3898.38	4329.68
03	1659876.00	4115.68	3701.52	3798.30	4249.28
04	1687986.00	4046.01	3649.13	3697.31	4227.86
05	1693897.00	4052.05	3720.50	3820.50	4366.29
06	1753784.00	4151.00	3690.26	3755.64	4233.69
Mean	1693718.00	4103.92	3651.59	3797.21	4286.22
Standard deviation	32404.45	77.46	69.89	67.44	57.30
% Relative standard deviation	1.91	1.89	1.91	1.78	1.34

Table 8. Method precision data for Celecoxib and its impurities

Injection	Celecoxib	4-methyl acetophenone.	Impurity-A	Impurity-C	Impurity-D	Percentage of 4-methyl acetophenone present in spiked sample	Percentage of impurity A present in spiked sample	Percentage of impurity C present in spiked sample	Percentage of impurity D present in spiked sample
1	97.88	98.17	96.71	100.42	100.57	0.238	0.208	0.225	0.254
2	100.90	103.08	99.05	102.66	101.01	0.250	0.214	0.230	0.256
3	98.00	100.29	101.37	100.03	99.14	0.243	0.219	0.224	0.251
4	99.66	98.59	99.93	97.37	98.64	0.239	0.215	0.218	0.250
5	100.01	98.74	101.89	100.61	101.87	0.239	0.220	0.226	0.258
6	103.55	101.15	101.06	98.91	98.77	0.245	0.218	0.222	0.250
Mean	100.00	100.00	100.00	100.00	100.00	0.242	0.216	0.224	0.253
Standard deviation	1.91	1.72	1.75	1.62	1.22	0.004	0.004	0.004	0.003
% Relative standard deviation	1.91	1.72	1.75	1.62	1.22	1.723	1.747	1.621	1.220

Precision

System precision

Results of system precision were reported in the table 4.11. Percentage relative standard deviation of system precision reports was with in 2. From the results, the method has a good system precision. Chromatogram of system precision was present in figure 4.16.

Method precision

Method precision results were given in percentage content. The individual results of eprosartan and its impurities were reported in the table 4.12.

DISCUSSION

Validation was performed on the developed analytical method

for its acceptable performance to ensure suitability of indent purpose. The validation parameters like accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, ruggedness and robustness were executed and established method conditions to meet the requirements to execute the analysis of celecoxib and its impurities. Under the specificity experiment samples were stressed various stress conditions and analyzed along with unstressed samples. Celecoxib was found to be very stable under all degradation conditions. The developed method can be used for routine analysis because the linearity found in Celecoxib, 4-methyl acetophenone, Impurity A, Impurity C and Impurity D was nearing 1 that is 0.9991, 0.9986, 0.9990, 0.9992 and 0.9990 respectively which shows the good regression for linearity. The results from solution stability experiments confirmed that standard and sample solutions were stable up to 24 h for both assay and related substances analysis. Maximum recovery is obtained by this developed method and the mean percentage recovery for each component was nearing 100%. Data of repeat experiment were showed <2% RSD (relative standard deviation) for assay and <2% RSD for impurities. In all the deliberate varied chromatographic conditions like flow rate (±0.2 mL/min), column temperature (±5°C), composition of organic solvent (±10% of method organic solvent) and pH of mobile-phase buffer (±0.2), all analyte and impurities were adequately resolved and elution orders remained unchanged. The resolution between all pair compounds was >2.0.These results are conforming good precision of the method. Therefore this method can be used for the routine analysis and one most important reason is that the developed method does not involve the use of expensive reagents. Also, our proposed method requires less time for the determination of Celecoxib and its known impurities simultaneously when compared to other methods. The developed method is uncomplicated, accurate, sensitive and precise for the determination of related substances in the Celecoxib. The satisfying % recoveries and low % RSD Values were confirmed the suitability of the developed method for the usual analysis of Celecoxib related substances in pharmaceuticals.

CONCLUSION

A validated HPLC analytical method has been developed for the determination of Celecoxib in bulk and dosage form. The proposed method was simple, accurate, precise, specific and suitable to use for the routine analysis of Celecoxib in either bulk API powder or in pharmaceutical dosage forms. Method validation parameters results are evaluated and found to be acceptable and this stability indication method can be used for regular analysis.

ACKNOWLEDGEMENT

CONFLICT OF INTERESTS

Declared None.

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