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DESIGN, CHARACTERIZATION AND IMPURITY PROFILING OF CELECOXIB BY RP-HPLC

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ABSTRACT

Celecoxib is one of the most widely used drug around the world. There are various pharmacological activities reported for the wonder moiety. Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, acute pain and primary dysmenorrhoea. The most common side effects upon usage of Celecoxib include indigestion, diarrhoea and abdominal pain. Celecoxib produces its therapeutic effects by inhibiting cyclooxygenase (COX), an enzyme which is responsible for the production of prostaglandins. Celecoxib, in addition has other pharmacological activities that are entirely independent of its COX-2 inhibitory activity. This selectivity allows Celecoxib and other COX-2 inhibitors to reduce inflammation (and pain) while minimizing gastrointestinal adverse drug reactions (e.g. stomach ulcers) that are common with non-selective NSAIDs. The present study is based on the synthesis and impurity profiling. A new isocratic RP-HPLC method was developed for the separation and determination of process related impurities in Celecoxib and validated as per ICH guidelines. The method was found to be simple, sensitive, precise, robust and accurate. Therefore, this method can be used for routine testing as well as stability analysis of Celecoxib drug substance. All statistical results (Mean, % RSD and % recovery) were within the acceptance criteria.

KEYWORDS: Celecoxib, pharmacological activity, chromatographic techniques, impurity profile.

INTRODUCTION

Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, acute pain and primary dysmenorrhoea. It acts as an anti-inflammatory, analgesic and antipyretic drug and also reduces the number of adenomatous colorectal polyps in FAP (Familial Adenomatous Polyposis). The most common side effects upon usage of Celecoxib include indigestion, diarrhoea and abdominal pain.[1-4] Celecoxib produces its therapeutic effects by inhibiting cyclooxygenase (COX), an enzyme which is responsible for the production of prostaglandins. There are two iso forms of COX.COX-1 and COX-2 which differ in substrate and inhibitor selectivity. Celecoxib is a selective inhibitor of COX-2 produced through the mediation of inflammatory ligands such as cytokines.[5] In contrast the traditional NSAID's inhibit both isoforms of cyclooxygenase and inhibit platelet aggregation.[6] This selectivity allows Celecoxib and other COX-2 inhibitors to reduce inflammation (and pain) while minimizing gastrointestinal adverse drug reactions (e.g. stomach ulcers) that are common with non-selective NSAIDs.[7] Celecoxib is approximately 10-20 times more selective for COX-2 inhibition over COX-1 where it binds with its polar sulfonamide side chain to a hydrophilic side pocket region close to the active COX-2 binding site.[8] Due to its specificity for the COX-2 inhibition, it has the potential to cause less gastropathy and risk of GI bleeding.[9-11] Both the isoforms of cyclooxygenases are involved in the production of prostaglandins whereas in addition COX-1 synthesizes thromboxane. Hence, inhibition of COX-2 leads to diminished prostaglandin synthesis without affecting thromboxane and thus has no effect on platelet aggregation or blood clotting.[12] Celecoxib, in addition has other pharmacological activities that are entirely independent of its COX-2 inhibitory activity.[13]

Procurement of API

Pfizer markets Celecoxib under the brand name Celebrex. Celecoxib is not currently available as a generic in the United States, because the intellectual property is still controlled by Pfizer. However, in other countries, including India and the Philippines, it is legally available as under the brand names Cobix and Celcoxx.[12]

Literature survey

Literature survey revealed that only a few analytical methods for determination of Celecoxib in pharmaceuticals as well as biological fluids such as blood, urine, serum and plasma were reported.

Rose et al. have reported a normal phase high-performance liquid chromatography method for the determination of Celecoxib in human plasma by UV detection.[14] Srinivasu et al. reported a validated LC method for the quantitative determination of Celecoxib in pharmaceutical dosage forms and purity evaluation in bulk drugs. In this method, separation was achieved by Novapak C18 column (300x3.9 mm) with 4 µm(particle size) and mobile phase consisting potassium dihydrogen phosphate buffer and acetonitrile with pH 4.8, the detection being made at 252 nm.[15] Srinivasu et al. developed a method for the determination of Celecoxib in pharmaceutical dosage forms by MEKC.[16] Saha et al. have developed a liquid chromatographic method for the determination of Celecoxib in pharmaceutical formulations using UV spectrophotometry.[17] Schonberger et al. reported a HPLC method for the determination of Celecoxib in human serum with fluorescence detection.[18] Chow et al. developed a HPLC method for the determination of Celecoxib in human plasma.[19] Zhang et al. have used HPLC method for the determination of Celecoxib in human plasma and breast milk.[20] Zarghi et al. have described a simple and rapid high-performance liquid chromatographic method for determination of Celecoxib in plasma using UV detection.[21]

So far there is no method reported for the determination of process-related substances of Celecoxib in the literature. The present study is aimed at developing a reversed phase HPLC method to determine the process-related impurities originating from the starting materials and intermediates of Celecoxib in bulk drugs. Forced degradation studies of CXB were carried out under thermal, photo, acidic, basic and peroxide conditions. A comprehensive study was undertaken to characterize process impurities by FT-IR, MS and ^{1}H NMR spectroscopy. The study protocols and details are given in following pages.

MATERIALS AND METHODS

Instrumentation

High performance liquid chromatography

An integrated HPLC system with computer based chromatography software (Empower) was used. The Waters alliance system with 2695 quaternary low pressure gradient system auto sampler, column thermostat and photodiode array detector was used for this experiment.

Chemicals and reagents

Celecoxib working standard and its process related impurities were synthesized at Pharmazell R&D Centre, Visakhapatnam (India) and obtained as gift samples. HPLC grade acetonitrile was obtained from Merck. Analytical grade dipotassium hydrogen phosphate and

orthophosphoric acid were used. High purity water was prepared by using Milli-Q Elix and then using Milli-Q academic purification system (Milli-pore). 4-Methyl acetophenone (impurity-A) was purchased from SL Drugs & Pharmaceuticals.

Preparation of Buffer

1.74 g of dipotassium hydrogen phosphate was dissolved in 1000 ml of water and pH was adjusted to 3.5 ± 0.05 with orthophosphoric acid. Then the solution was filtered through 0.45 µ filter paper and degassed in ultrasonic bath.

Preparation of mobile phase and diluent

A mixture of the buffer and acetonitrile in the ratio 45:55% v/v was prepared and degassed. Mobile phase was used as diluent.

Preparation of Standard solution

Standard solution was prepared by weighing accurately 50 mg of Celecoxib and transferring into 50 ml volumetric flask containing 30 ml of diluent. The contents of flask were sonicated for 15 min. to dissolve the drug completely and the volume was made up to 50 ml with the diluent (1 mg/ml).

Preparation of test solution

About 50 mg of sample was weighed accurately and transferred into 50ml volumetric flask, 30 ml of diluent was added, sonicated for 15 min. to dissolve the drug completely and the volume was made up to 50 ml with diluent.

Preparation of impurity mixture

About 37.5 mg of Celecoxib and its process related impurities (impurity A, B, C and D) were weighed accurately and transferred into 100 ml volumetric flask, 60 ml of diluent was added to dissolve the compounds and the volume was made up to 100 ml with diluent. 5 ml of this solution was made up to 50 ml with diluent to get a concentration of 37.5 µg/ml. This impurity stock solution was adequately diluted to study accuracy, precision, linearity, limit of detection and limit of quantitation.

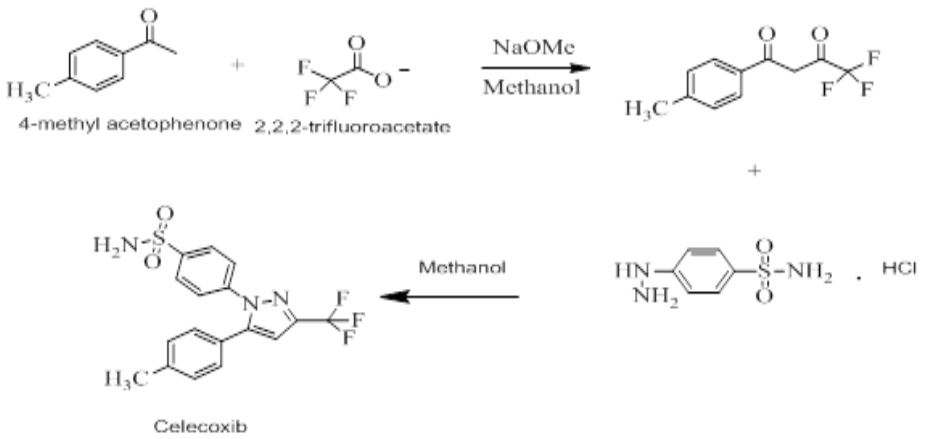
Method Development and Optimization of Celecoxib by RP-HPLC

In order to develop a suitable and robust RP-HPLC method for the determination of Celecoxib and its process related impurities, an attempt was made using a C18 column and mobile phase composed of phosphate buffer and methanol in the ratio 60:40% v/v. In this

mobile phase, peak symmetry is not satisfactory, peaks are splitting and separation was also not good. Therefore organic modifier concentration was changed to 40-50% but no improvement was observed. Many experiments were conducted using different columns, different buffers and different organic modifier concentrations. Even then no improvement was observed and hence, methanol was replaced with acetonitrile. Some improvement was observed but the impurity peaks were merging with Celecoxib peak. So further trails were carried out by varying the pH and concentration of buffer and acetonitrile. Eventually a mobile phase composed of phosphate buffer (pH 3.5) and acetonitrile in the ratio 45:55% v/v gave sharp peaks with minimum tailing and good resolution for both the drug and impurities. The optimized chromatographic conditions are shown in Table 3.1.

Table 3.1 Optimized chromatographic conditions

Stationary phase (column)	Inertsil ODS C18 (250x4.6 mm),5µm
Mobile phase	Phosphate Buffer: Acetonitrile 45:55% v/v
Flow rate (ml/min)	1.0 ml/min
Column temperature (ºC)	25ºC
Volume of injection (µl)	20 µl
Detection wavelength	250 nm

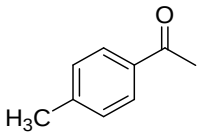
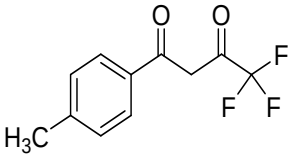
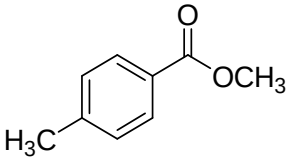
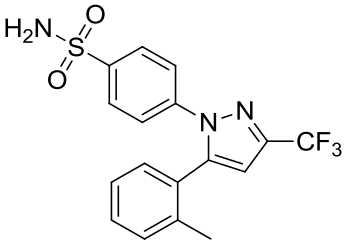


Synthesis of Celecoxib

Process-related impurities

The process-related impurities that may appear in the final API of Celecoxib are listed in Table 3.2.

Table 3.2 Process-related impurities for Celecoxib

S. No	Name of the Impurity	Structure	Impurity Code
1	4-Methyl acetophenone		Impurity-A
2	Trifluroacetyl 4-Methyl acetophenone		Impurity-B
3	Methyl-4-methyl benzoate		Impurity-C
4	4-[5-(2-methylphenyl) -3-(trifluoromethyl) -1H-pyrazol-1-yl]-benzene sulphonamide		Impurity-D

The process-related impurities in the API "Celecoxib" were identified using the standards provided by Pharmazell R&D Centre, India (Pvt.) Ltd. These impurities were synthesized and characterized before using them for this study. The impurities were injected into the chromatographic system separately and later combining with Celecoxib (spiked with sample). The impurities in the drug substance were identified based on the retention time (RT) and relative retention time (RRT) observed from the spiked study. The data was given in Table 3.3 and their individual chromatograms were shown in the Fig 3.1-3.6.

Table 3.3 RT and RRT for Celecoxib and its impurities

S. No	Name	RT (min)	RRT(min)
1.	Impurity-A	8.02	0.44
2.	Impurity-B	7.46	0.41
3.	Impurity-C	11.82	0.66
4.	Impurity-D	16.14	0.90
5.	Celecoxib	17.98	1

Typical chromatograms for individual impurities

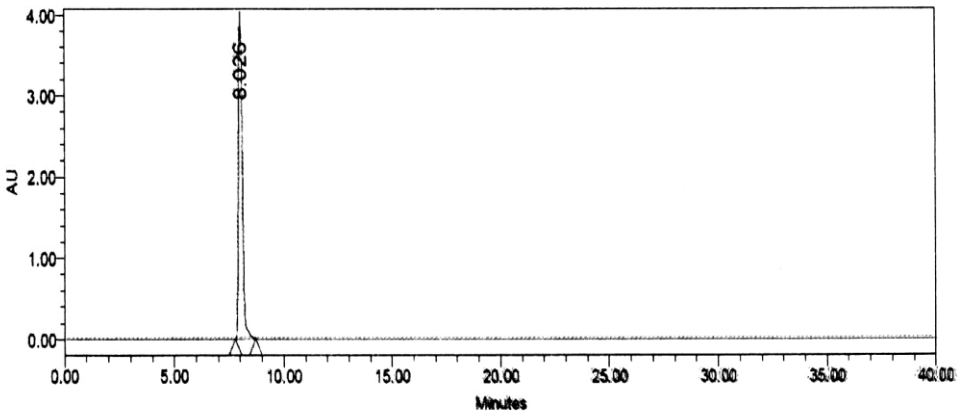


Fig 3.1 Typical chromatogram for impurity-A

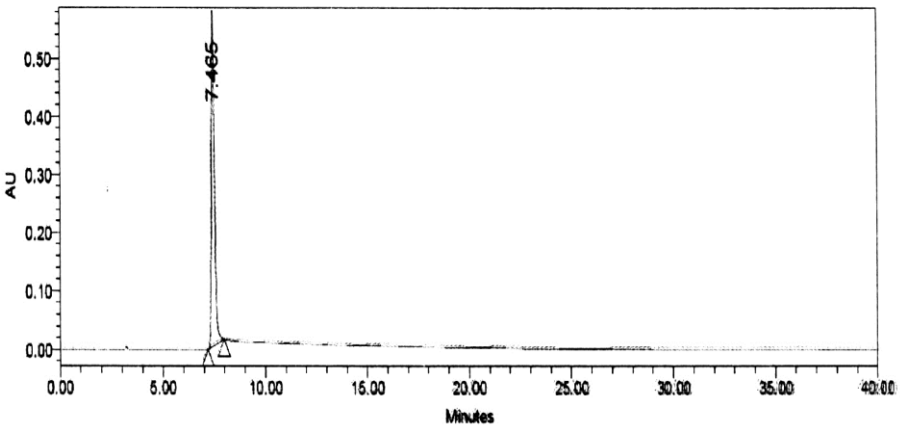


Fig 3.2 Typical chromatogram for impurity-B

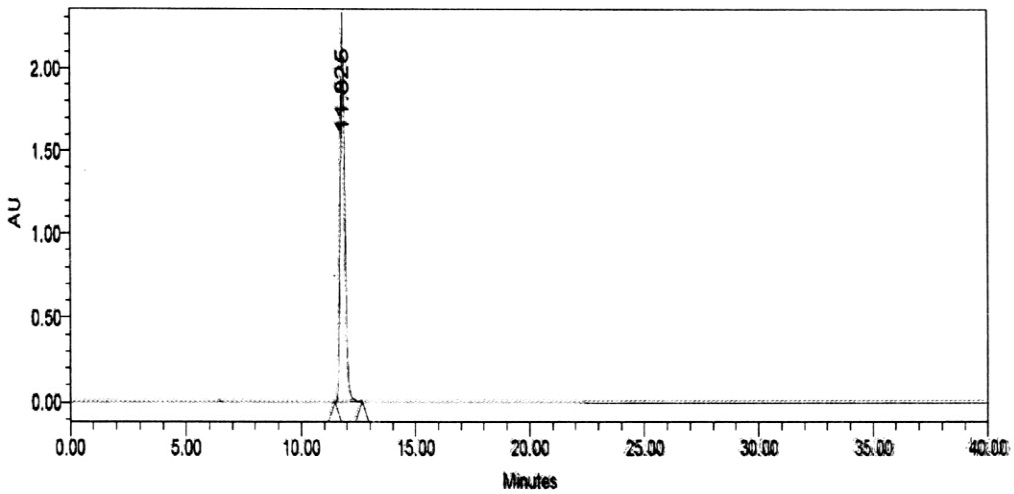


Fig 3.3 Typical chromatogram for impurity-C

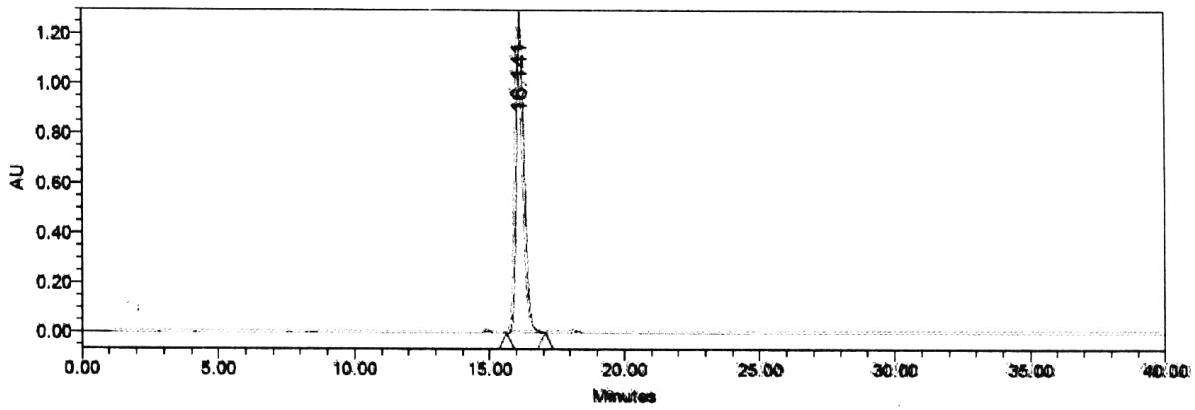


Fig 3.4 Typical chromatogram for impurity-D

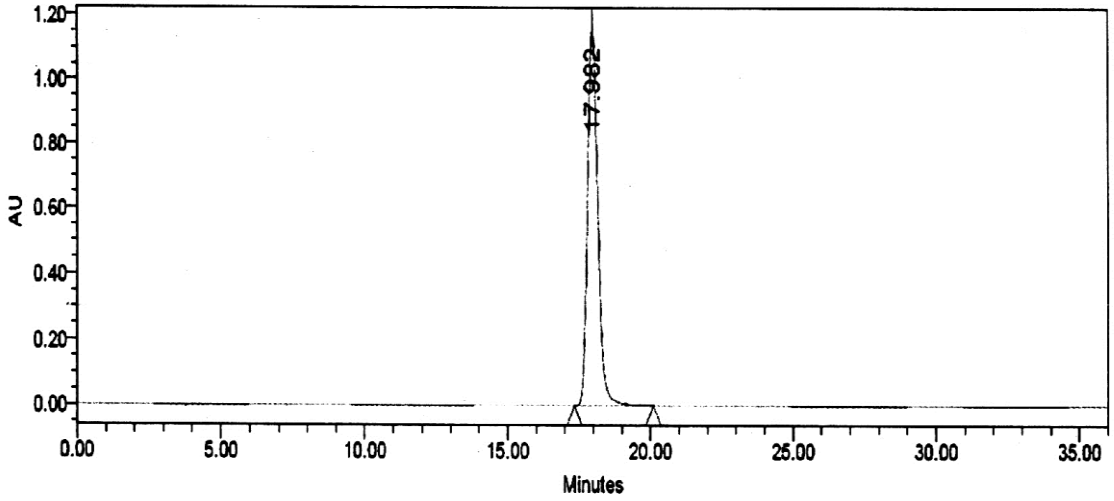


Fig 3.5 Typical chromatogram for Celecoxib

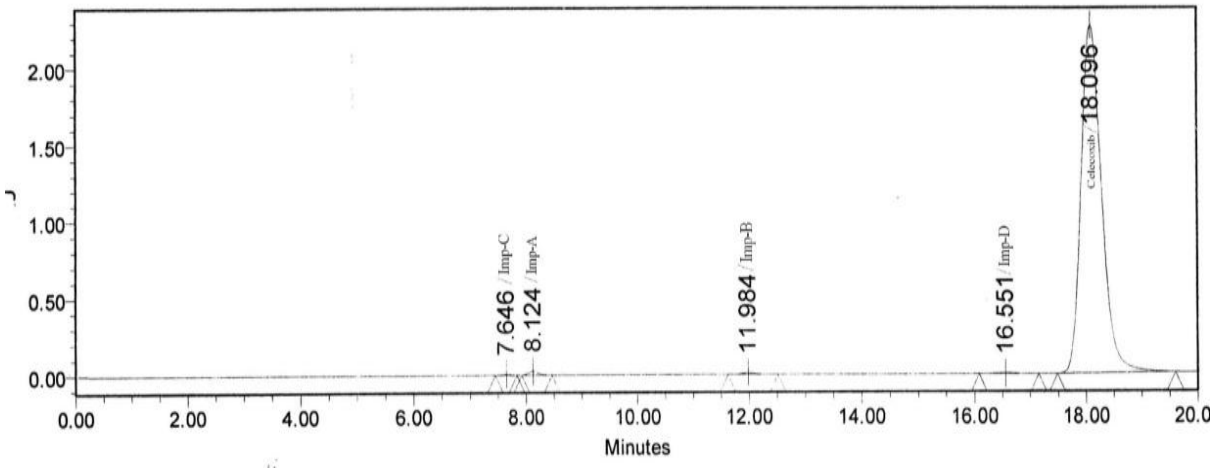


Fig 3.6 Typical chromatogram for Celecoxib spiked with impurities Celecoxib

Structural confirmation of Celecoxib

Celecoxib was analyzed by spectral techniques using IR, ^{1}H NMR and MS for structural confirmation.

FT-IR

The FT-IR spectrum was recorded in the solid state as KBr dispersion medium using Perkin-Elmer 100 instrument. The IR spectral data was given in Table 3.10 and its corresponding spectrum was shown in Fig 3.16.

Table 3.10 IR assignments for Celecoxib

S.No	Feature	Wave number (cm^{-1})
1.	C-H (-CH_3)	2869.03
2.	C-H (Aromatic)	3099.49
3.	-CF_3	762.80, 794.16, 845.68
4.	O=S=O	1345.03, 1164.01
5.	C=C (Aromatic)	1595.69, 1563.43
6.	N-H	3342.28
7.	C-N	1230.34

^{1}H NMR

The ^{1}H NMR studies were performed on Avance-300 MHz NMR spectrometer in $CDCl_3$. ^{1}H chemical shift values are reported on the δ scale in ppm. NMR assignments of Celecoxib are given in Table 3.11 and its corresponding spectrum was shown in Fig 3.17.

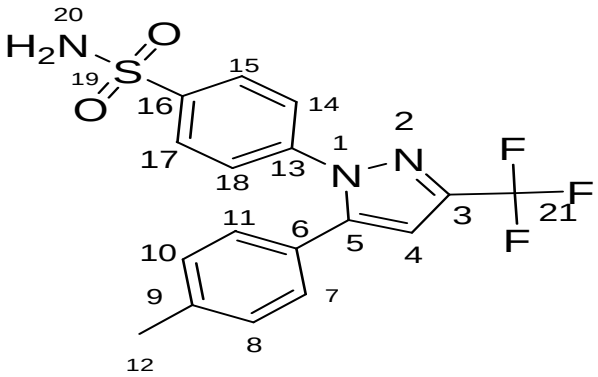


Fig 3.16 IR Spectrum for Celecoxib

Table 3.11 ^{1}H NMR assignments for Celecoxib

Position	^{1}H	δ (ppm)	Splitting
4	1H	6.7	d
7	1H	7.1	d
8	1H	7.3	d
10	1H	7.3	d
11	1H	7.1	d

12	3H	2.4	s
14	1H	7.5	d
15	1H	7.8	d
17	1H	7.8	d
18	1H	7.8	d
20	2H	4.8	s

Fig 3.17 ^{1}H NMR Spectrum for Celecoxib

Mass spectral data

The electron impact ionization mass spectrum showed (Fig 3.18) characteristic fragmentation pattern of the sample. The sample was introduced into the source with the help of a particle beam interface. The molecular ion $[M]^{+}$ peak of Celecoxib (4-[5-(4-Methylphenyl)-3-(trifluoromethyl) pyrazol-1yl] benzene sulfonamide) was observed at m/z 382. From the above spectral data, the structure was confirmed as 4-[5-(4-Methyl phenyl)-3-(trifluoromethyl) pyrazol- 1yl] benzene sulfonamide.

Forced degradation study

Stability testing of an active substance or finished product provide evidence on how the quality of a drug substance or drug product varies with time influenced by a variety of environmental conditions like temperature, humidity and light etc,. Knowledge from stability studies enables understanding of the long-term effects of the environment on the drugs. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation path ways of drug as well as interaction between the drug and the excipients in drug product.

Forced degradation study was carried out by treating the sample under the following conditions

Acid degradation

50 mg of sample was weighed and transferred into 100 ml volumetric flask and 5 ml of 0.1N HCl was added to it. The solution was warmed on a water bath at 80°C for 24 hrs and then neutralized with 5 ml of 0.1N NaOH. The neutralized solution was made up to the volume with diluent.

Alkali degradation

50 mg of sample was weighed and transferred into 100 ml volumetric flask and 5 ml of 0.1N NaOH was added to it. The solution was warmed on a water bath at 80°C for 24 hrs and

then neutralized with 5 ml of 0.1N HCl. The neutralized solution was made up to the volume with diluent.

Thermal degradation

200 mg of the sample was taken in a watch glass and kept in an oven at 105°C temperature for 24 hrs. From that sample 50 mg was accurately weighed and transferred into 100 ml volumetric flask, dissolved and the was volume adjusted with diluent.

Humidity degradation

200 mg of the sample was left at room temperature for 24 hrs. From that sample 50 mg was accurately weighed and transferred into 100 ml volumetric flask, dissolved and the volume adjusted with diluent.

Photolytic degradation

200 mg of the sample was exposed to UV light under 254 nm for 24 hrs by using photo stability chamber. From that sample 50 mg was accurately weighed and transferred into 100 ml volumetric flask, dissolved and the volume adjusted with diluent.

Oxidative degradation

50 mg of the sample was weighed and transferred into a 100 ml volumetric flask and 5 ml of 5% potassium permanganate solution was added to it. The solution was warmed on water bath at 80°C for 3 hrs. Then the above mixture was kept aside for few minutes, and the volume was made up with diluent.

The above stressed samples were analyzed as per the test procedure using Photodiode Array detector. The results are summarized in Table 3.12 and its chromatograms are shown in Fig 3.19.

Table 3.12 Results of Forced degradation study

Stress condition	Purity angle	Purity threshold	Assay (%)	Degradation
Acid degradation	0.490	11.523	98.7	No degradation peak was observed
Alkali degradation	0.470	12.062	98.4	No degradation peak was observed
Thermal degradation	0.186	0.663	99.4	No degradation peak was observed
Humidity degradation	0.105	0.552	99.7	No degradation peak was observed
Photolytic degradation	0.140	0.636	99.5	No degradation peak was observed

Oxidative degradation	0.196	9.541	82.3	Observed significant degradation about 18%. The major degradation peaks are at 6.2 min. The degradation peaks are well resolved from main peak and also from known impurity peak. This degradation peaks are not matching with any of the known peaks

Note: If the purity angle is less than the threshold angle, it is said to have passed the purity test.

Acceptance criteria

In any one of the identified stress conditions, the drug product should degrade to 10-20%.

RESULT

18.0% degradation observed with KMnO4

Examine the peak purity for Celecoxib. It was found to be spectrally homogenous and passed the purity test. (In Waters HPLC, the peak purity for Celecoxib was examined).

CONCLUSION

The above forced degradation study showed that Celecoxib undergone significant degradation only in the presence of potassium permanganate .The oxidation degradation peaks were separated well from the main peak. Peak separation, peak purity results showed that the method is specific and capable of picking up all the degradation peaks. Hence, it was concluded that the method was very selective and stability indicative and suitable for the determination of impurities in the pure drug.

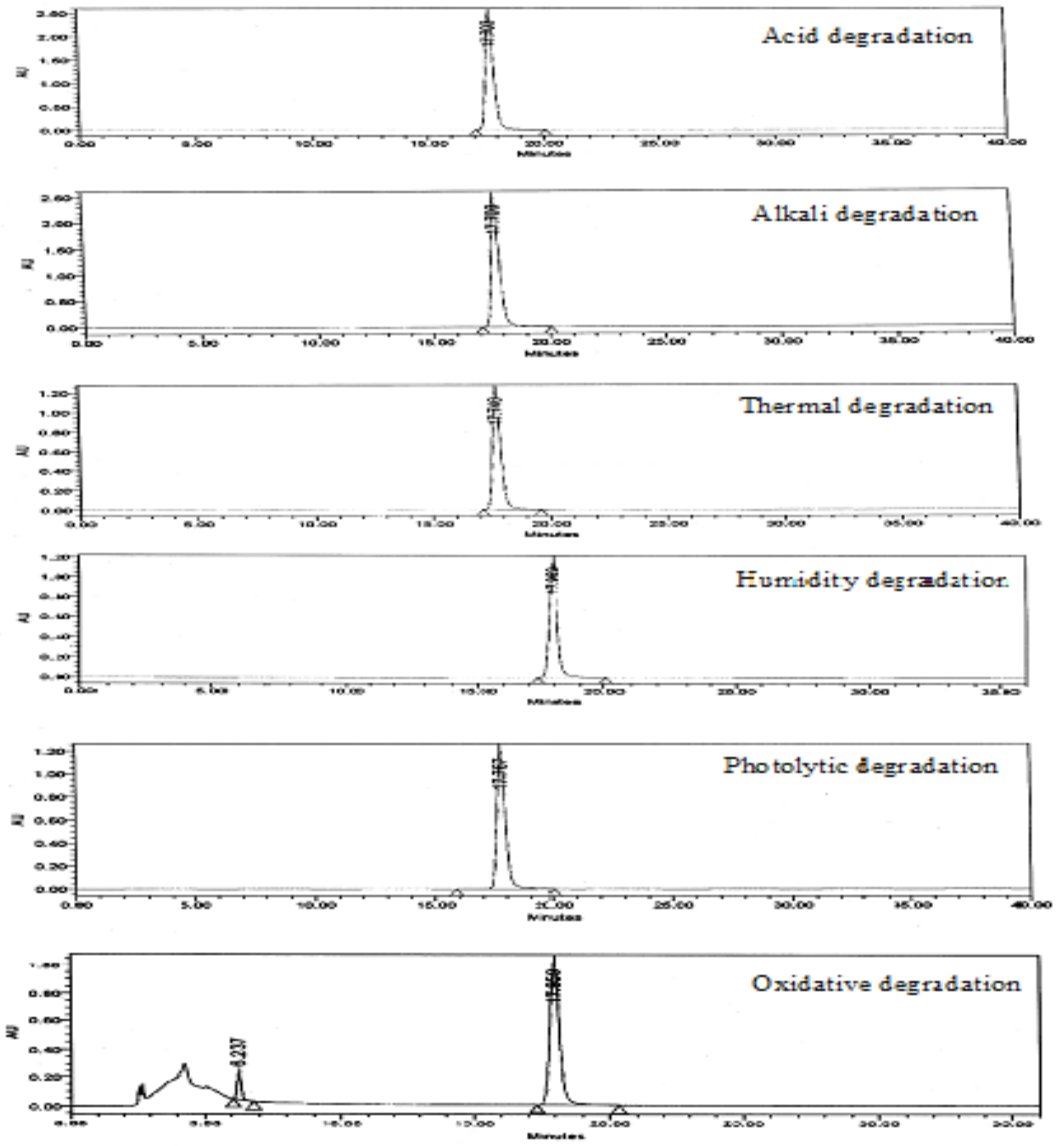


Fig 3.19 Forced degradation chromatograms for Celecoxib

Method Validation

Analytical method validation is a process that demonstrates the suitability of the proposed procedures for the intended purpose. More specifically, it is a matter of establishing documented evidence providing a high degree of assurance with respect to the consistency of the method and results. It evaluates the product against defined specifications. The validation parameters viz., specificity, accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and system suitability have to be evaluated as per the ICH guidelines for all analytical methods developed by HPLC.

Validation Characteristics

The following validation characteristics were verified as per the ICH guidelines.

- System suitability
- Specificity
- Linearity
- Accuracy
- Precision
- LOD & LOQ
- Robustness

System Suitability

Parameters such as plate number (N), asymmetry or tailing factors (A_s), relative retention time (RRT), resolution (R_s) and reproducibility (%R.S.D), retention time were determined (Table 3.13). These parameters were determined during the analysis of a "sample" containing the main components and related substances. System suitability terms were determined and compared with the recommended limits ($1 \geq A_s \leq 2$ and $R_s > 1.5$).

Table 3.13 System suitability data

Name	RT	RRT			
			Resolution(R_s)	Theoretical plate(N)	Peak symmetry(A_s)
Impurity-A	8.07	0.45	-	11424	1.11
Impurity-B	11.89	0.66	10.5	13534	1.07
Impurity-C	7.59	0.42	1.57	10069	1.07
Impurity-D	16.32	0.91	8.86	12702	1.05
Celecoxib	17.87	1	2.53	13017	1.04

Specificity

The specificity of the developed HPLC method was performed by injecting blank solution and standard solution spiked with process-related impurities separately. The chromatogram of drug with impurities was compared with the blank chromatogram, to verify the blank interference. No peak was observed at the retention time of Celecoxib and its impurities. Hence, the method is specific for the determination of process related impurities in Celecoxib.

Linearity

Standard solutions at different concentration levels ranging from LOQ to 2.25 µg/ml (150% of specification limit) were prepared and analyzed in triplicate. In order to demonstrate the linearity of detector response for Celecoxib and its impurities, the linearity plot was drawn taking the concentration on X-axis and the mean peak area on Y-axis. The data were

subjected to statistical analysis using a linear-regression model. The regression equations and correlation coefficients (r^2) are given in Tables 3.14-3.18 and their linearity plots are shown in Fig 3.20-3.24.

Acceptance criteria

The Correlation Coefficient should not be less than 0.99.

Table 3.14 Linearity data for impurity-A

S.No	Concentration (µg/ml)	Peak area(N=3)	Mean peak area
1	0.378	55878 55446 55501	55608
2	0.756	106924 108443 108757	108041
3	1.134	166261 168731 166237	167076
4	1.512	216146 220118 218069	218111
5	1.89	264653 268764 265986	266468
6	2.268	307639 309746 307450	308278
Correlation coefficient		0.9972	
Slope		135273	
Intercept		8297.1	

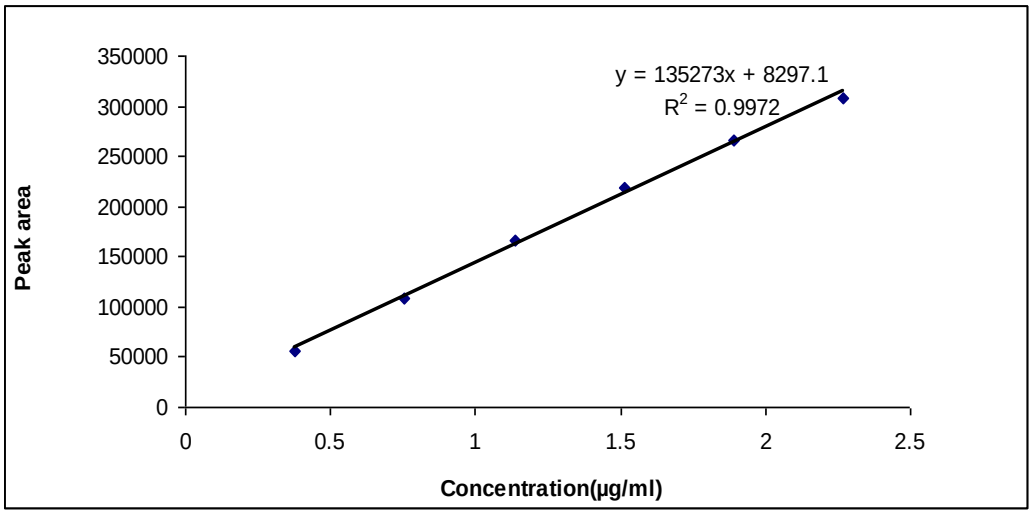


Fig 3.20 Linearity plot for impurity-A

Table 3.15 Linearity data for impurity-B

S.No	Concentration (µg/ml)	Peak area(N=3)	Mean peak area
1	0.373	26918 26923 27057	26966
2	0.746	55019 45918 54965	54967
3	1.119	84598 85537 85989	85374
4	1.492	110591 113575 113300	112488
5	1.865	131425 130643 130769	130946
6	2.238	157938 158703 156587	157742
Correlation coefficient		0.9953	
Slope		69623	
Intercept		3854.1	

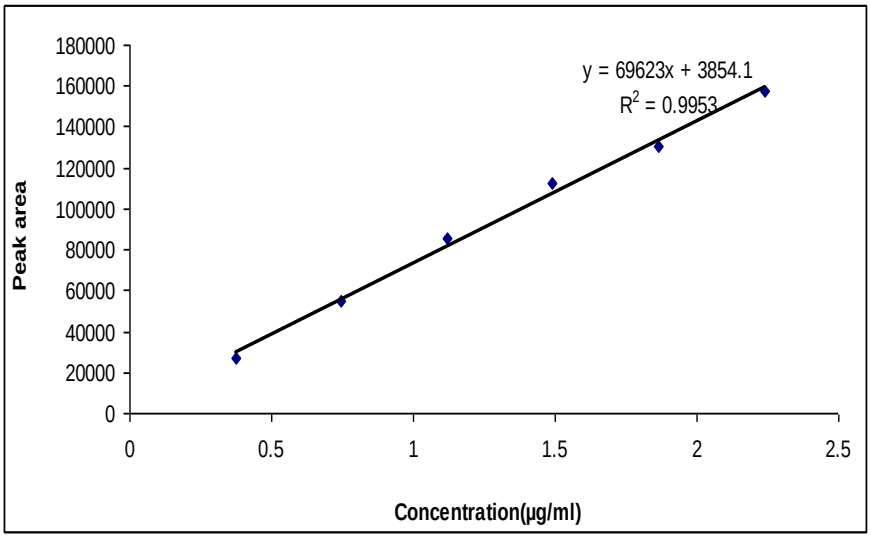


Fig 3.21 Linearity plot for impurity-B

Table 3.16 Linearity data for impurity-C

S.No	Concentration (µg/ml)	Peak area(N=3)	Mean peak area
1	0.373	10912 11191 11331	11114
2	0.746	21920 20838	20825

		19717	
3	1.119	34657 30930 34074	33220
4	1.492	45795 46397 46056	46082
5	1.865	56893 55032 55637	55854
6	2.238	65796 62677 64112	64195
Correlation coefficient		0.9954	
Slope		29365	
Intercept		212.93	

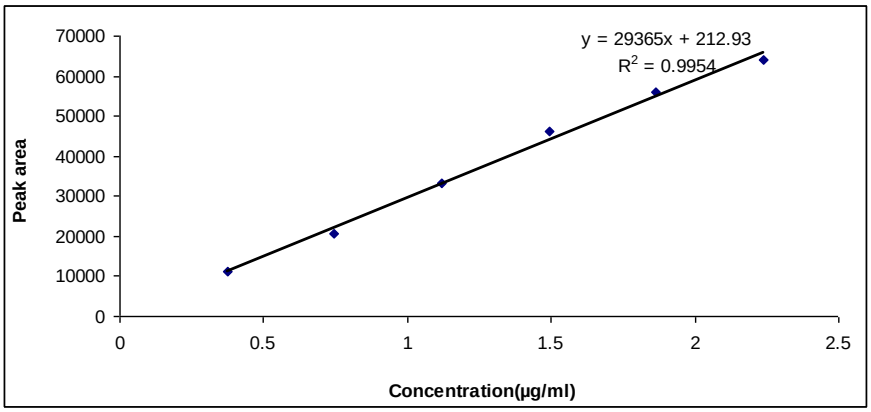


Fig 3.22 Linearity plot for impurity-C

Table 3.17 Linearity data for impurity-D

S.No	Concentration (µg/ml)	Peak area(N=3)	Mean peak area
1	0.377	19649 18662 19871	19727
2	0.754	38897 37719 37934	38183
3	1.131	59584 57398 60850	59277
4	1.508	78105 79018 77695	78301
5	1.885	98342 99231 98346	98639

6	2.238	111087 111030 110952	111023
Correlation coefficient		0.9997	
Slope		52505	
Intercept		-557.2	

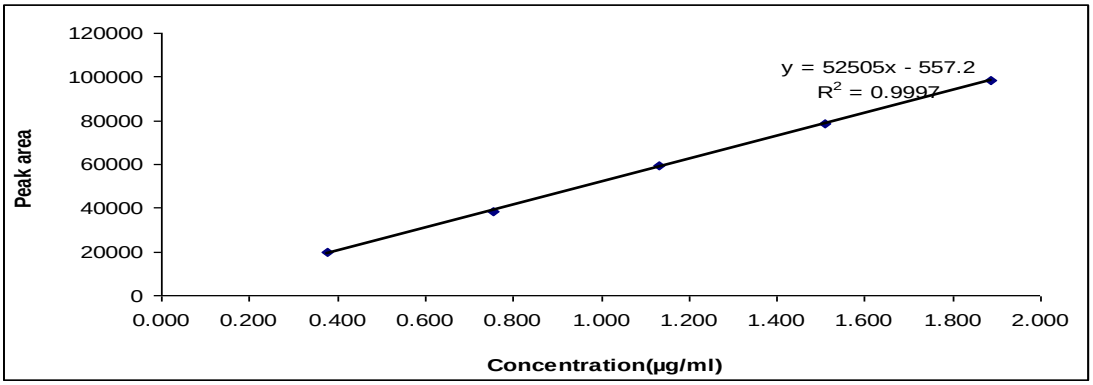


Fig 3.23 Linearity plot for impurity-D

Table 3.18 Linearity data for Celecoxib

S.No	Concentration (µg/ml)	Peak area(N=3)	Mean peak area
1	0.374	29979 29670 29843	29831
2	0.748	58672 58241 58632	58515
3	1.122	89848 89493 89426	89589
4	1.496	119345 118768 118896	119003
5	1.870	148987 148446 147987	148473
6	2.244	176645 176876 175987	176503
Correlation coefficient		0.9998	
Slope		78888	
Intercept		387.53	

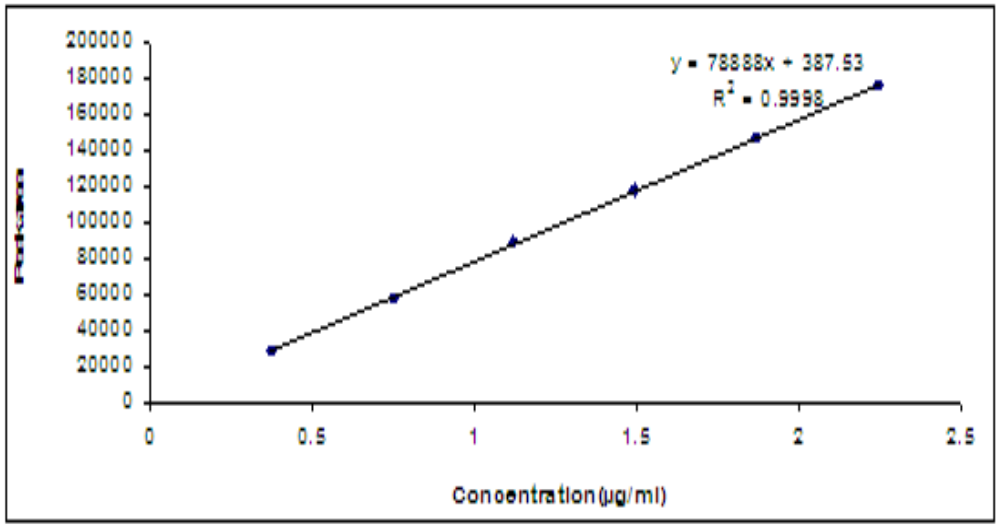


Fig 3.24 Linearity plot for Celecoxib

Accuracy/Recovery

Accuracy of the test method was determined by analyzing Celecoxib drug substance spiked with impurities at three different concentration levels of 50%, 100%, and 150% of each in triplicate at the specified limit. The mean recoveries of all the impurities were calculated (Table 3.19).

Table 3.19 Recovery studies for impurities of Celecoxib

Impurity name	Spike level (%)	Concentration spiked (µg/ml)	Concentration recovered(µg/ml)	% Recovery[a]
	50	0.76	0.73	96.92
Impurity-A	100	1.52	1.51	99.34
	150	2.28	2.18	95.76
	50	0.74	0.72	97.74
Impurity-B	100	1.48	1.44	97.52
	150	2.26	2.23	98.82
	50	0.66	0.63	95.38
Impurity-C	100	1.33	1.28	96.16
	150	1.99	1.93	97.07
	50	0.72	0.71	97.75
Impurity-D	100	1.45	1.41	97.29
	150	2.18	2.12	97.22

a; average of three determinations.

Acceptance criteria: The mean recovery of the impurities at each level should be not less than 85.0% and not more than 115.0%.

RESULT The % recovery obtained is well within the limit of 85% - 115%. This indicated that the method is accurate to determine the impurities in Celecoxib.

Precision

System precision of the method was evaluated by injecting the standard solution six times and percent relative standard deviation (% R.S.D) for area of Celecoxib peak was calculated. It was found to be less than 2.0% (R.S.D). The precision of the method for the determination of impurities related to Celecoxib was studied for repeatability and intermediate precision at 100% level. Repeatability was demonstrated by analyzing the standard solution spiked with impurities for six times. The %R.S.D for peak area of each impurity was calculated. Intermediate precision was demonstrated by analyzing same sample of Celecoxib by two different analysts on two different days (Inter-day). Intra-day variations of impurities related to Celecoxib were expressed in terms of %R.S.D.Values. Repeatability and intermediate precision for the process-related impurities in Celecoxib were found to be less than 2.0% R.S.D. The results are given in Table 3.20, which confirmed good precision of the method.

Table 3.20 Precision studies for Celecoxib and its impurities

Name	Method precision % R.S.D(n=6)	Intermediate precision	
		Intra day % RSD(n=6)	Inter day % RSD(n=6)
Celecoxib	0.32	0.46±0.08	0.42±0.08
Impurity-A	0.98	0.92±0.03	0.89±0.07
Impurity-B	0.48	0.51±0.06	0.49±0.03
Impurity-C	0.74	0.72±0.04	0.69±0.06
Impurity-D	0.82	0.76±0.02	0.89±0.04

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by blank samples and calculating the signal-to-noise ratio for each compound by injecting a series of solutions until *S/N* ratio 3 for LOD and 10 for LOQ.

The LOQ and LOD values shown in Table 3.21, 3.22 and its chromatograms were shown in Fig 3.25, 3.26.

Table 3.21 Results for limit of quantitation

S. No	Name	Concentration (µg/ml)	Observed signal to noise ratio
1	Impurity-A	0.378	10.4
2	Impurity-B	0.373	9.9
3	Impurity-C	0.373	9.8
4	Impurity-D	0.377	9.6
5	Celecoxib	0.374	10.2

Table 3.22 Results for limit of detection

S. No	Name	Concentration (µg/ml)	Observed signal to noise ratio
1	Impurity-A	0.126	3.4
2	Impurity-B	0.124	3.2
3	Impurity-C	0.124	2.9
4	Impurity-D	0.125	3.1
5	Celecoxib	0.124	3.1

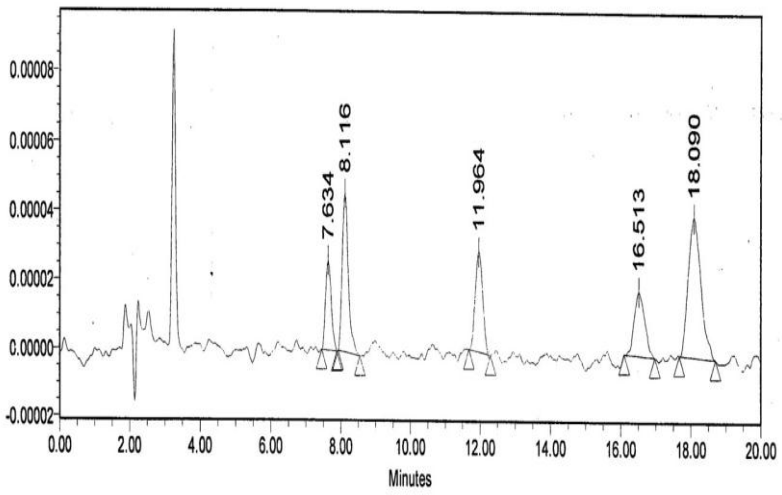


Fig 3.25 Typical chromatogram for LOQ

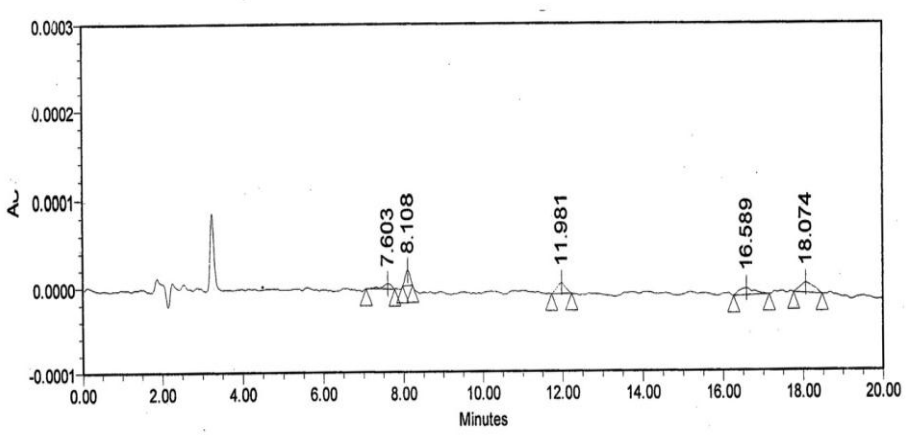


Fig 3.26 Typical chromatogram for LOD

Robustness

To determine the robustness of the developed method, chromatographic conditions were deliberately altered. The parameters selected were change in flow rate (± 0.2 ml/min), change in pH of the buffer (± 0.2) , change in the ratio of mobile phase (± 4%) and change in the column temperature (± 5ºC), the rest of the chromatographic conditions for each alteration study was kept constant.

RESULT

In all the deliberately varied chromatographic conditions, no significant change was observed, which confirmed the robustness of the developed method.

CONCLUSION

A new isocratic RP-HPLC method was developed for the separation and determination of process related impurities in Celecoxib and validated as per ICH guidelines. The method was found to be simple, sensitive, precise, robust and accurate. Therefore, this method can be used for routine testing as well as stability analysis of Celecoxib drug substance. All statistical results (Mean, % RSD and % recovery) were within the acceptance criteria.

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