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Determination of Assay for Active Pharmaceutical Ingredient Celecoxib by Chromatographic Technique

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ABSTRACT:
A simple, linear and reproducible assay method was developed for the quantification of Celecoxib API by using high performance liquid chromatography. The method was developed by using Universal C18, (100 x 4.6) mm, 5-micron column. Mobile phase consisted of Water: Methanol: Orthrophosphoric acid in ratio 38:62:0.1 with flow rate of 1.3 ml/min at 250 nm and column oven temperature at 40°C. RSD for standard preparation under system precision was observed 0.10%. RSD for retention time was observed 0.15% which shows reproducibility during replicate injections. The linearity range was achieved from 500 to 1500 ppm level for Celecoxib. Comparative data of method and intermediate precision shows average assay value 99.8% on as is basis. The method was applied for quantification of assay of Celecoxib API.

KEY WORDS: HPLC Assay, Celecoxib API.

I. INTRODUCTION:

CELEBREX (Celecoxib[1]) capsule is a nonsteroidal anti-inflammatory drug, available as capsules containing 50 mg, 100 mg, 200 mg and 400 mg Celecoxib for oral administration. The chemical name is 4-[5-(4-methylphenyl)- 3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide and is a diaryl-substituted pyrazole. The molecular weight is 381.38. Its molecular formula is $C_{17}H_{14}F_3N_3O_2S$. Celecoxib is a non-steroidal anti-inflammatory drug (NSAID). It is used for the treatment of osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms. It reduces numbers of colon and rectum polyps in patients with adenomatous polyposis. Oss C. and Dabre Rahul etal[2] worked for pre formulation studies for development of a generic capsule formulation of Celecoxib comparable to the branded (Reference) Product. Katravath et al.[3] worked for Celecoxib by reverse phase HPLC method.

II. MATERIALS AND METHODS:

HPLC grade methanol as a solvent (Spectrochem), orthrophosphoric acid AR grade (Rankem) and HPLC grade water (Merck) were used. Celecoxib test sample was received from Ramdev Chemical as a free sample. HPLC instrument of make Shimadzu LC 2010C HT with a quaternary gradient pump system and a fixed dual wavelength UV detector having LC-solution software with auto sampler tray with cooling facility and column oven temperature compartment is available was used for measurements.

Development of method was initiated by keeping aim to develop simple isocratic, easy setup of experimentation and cost-effective short run time assay quantification HPLC method for Celecoxib API, in such a way that we will get accurate and reproducible results in short period of time. The reference method evluated from Rao YM, et .al[4] and SinghaiS., et.al[5].

Current work presents assay quantification method by HPLC to quantify Celecoxib API against standard.

Short length, lower micron size C18 column was selected to reduce run time and to achieve excellent separation with sharp peak shapes. Because of short length compound eluted faster and C18 carbon load in column helps for separation and lower particle size column gives sharp peaks as well as more separation. Column oven temperature study was performed in order to reduce the backpressure of column ensuring that it does not impact on separation. Some basic compounds have property to get retained in column and because of such compounds get stuck inside the column and when they eluted it gives more tailing to peak. In this case higher column oven temperature increases kinetic energy of compound for early elution with good separation and symmetric peak shape will be obtained. Also, it reduces viscosity of mobile phase which helps to reduce the back pressure of column. In our method mobile phase is also easy to setup, which was premixed and degassed solution of HPLC grade water: Methanol: Orthrophosphoric

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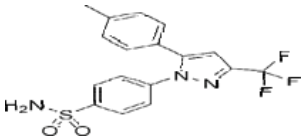
acid in ratio 38:62:0.1 was used. Run time was kept 10 minutes and 10 micro liter injection volume was injected to avoid excess of sample loading on column. Since excess sample loading on column baseline may get disturbed, hump and broad peak shape may not observed.

Mobile phase and other working solutions were filtered through 0.45-micron membrane filter and degassed by sonication**.** Slowly the flow rate was increased to achieve the column oven temperature and equilibrate column in mobile phase. Direct application of flow rate creates high pressure on column and may damage it. Diluent (water: methanol 90:10) was injected as blank preparation. No peak was observed in blank preparation at retention time of Celecoxib means there is no any blank interference. Five replicate injections of standard preparation were injected for calculation of % RSD.



4-[5-(methylphenyl)-3-(trifluoromethyl)-1H-pyrazole-1yl] benzene-1-sulfonamide[6-7]

Chromatographic conditions:

Column name: Universal C18(100x4.6)mm, 5 micron

Flow rate	:	1.3 ml/min
Wavelength	:	250 nm
Column Oven temperature	:	40°C
Run time	:	10 minutes.
Injection volume	:	10 µl

Mobile phase: Water: Methanol: Orthrophosphoric acid (38:62:0.1)

Diluent : Water: Methanol (90:10)

III. RESULTS AND DISCUSSION:

a) Chromatography:

Standard stock solution of Celecoxib was prepared by weighing accurately 50 mg of Celecoxib standard in 50 ml standard volumetric flask. 25 ml of diluent was added and sonicated to dissolve, and volume was made up to mark with water. Thus 1000 ppm of Celecoxib solution was prepared.

From the chromatogram (Fig. 1) it was concluded that the Retention time for Celecoxib was 2.65 ± 0.05 minutes and tailing factor was 1.30 which as per USP should not be more than 1.5.

Method development trials were continued for selection of mobile phase in order to retain the Celecoxib peak. In this study Methanol concentration was decreased in order for symmetric of peak and to reduce the run time which is our predefined aim. By this Methanol composition optimized and found that 0.1% orthophosphoric acid in Water and Methanol in ratio 38:62 as a mobile phase. In this condition satisfactory peak was observed at about 2.70 mins and also unknown peak found separated at retention time about 4.73 minutes.

During forced degradation study, test sample was treated by adding 5 ml, 5 N hydrochloric acid solutions, kept the solution at room temperature for 1 hr, and then analyzed. Then also, formation of degradation impurities found well separated from Celecoxib peak and method found suitable in terms of acid degradation sample. During acid degradation study unknown impurity at retention time about 0.97 and 1.71 minute is found as degradation impurity because in test sample these impurities are not detected and in acid degradation sample this impurity increases up to 1.6% and 0.91% respectively. In addition, acid degradation blank was prepared and injected. It was found that there is no interference from blank to any peak.

In alkali forced degradation study, test sample was treated by adding 5 ml, 5 N Sodium hydroxide solutions and kept it for 60 min at room temperature. After that, it was analyzed. It was observed that Celecoxib peak was slightly degraded and there was a formation of unknown impurity at retention time 2.78 minutes. However, the percentage of this degradation impurity is very less i.e. 0.44%. Hence, for determination of more degradation impurities test sample was treated by adding 5 ml, 5 N Sodium hydroxide solution and kept it for 12 hrs at room temperature and then analyzed. Then it was observed that Celecoxib peak was degraded and there is a formation of unknown impurity at retention time 2.78 minutes and formation of impurity is 5.03%

Table- 1: System precision

Sr.No	RT (min)	Area
1	2.761	7547024
2	2.764	7538672
3	2.763	7539123
4	2.768	7527813
5	2.771	7530300
Average	2.76	7536586
% RSD	0.15	0.10

The linearity solutions were prepared with five concentration levels i.e. 501.92, 752.88, 1003.84, 1254.80 and 1505.76 ppm of Celecoxib. The

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response of the drug is found to be linear in the investigation concentration range. The correlation coefficient was 0.9999. Graphs obtained during linearity study is shown in Figure no 7. Celecoxib sample is chemically treated by adding 5 ml, 5 N hydrochloric acid solution and 5 N sodium hydroxide solution and kept at room temperature for 1 hr separately and then analyzed. It is observed that in newly developed method Celecoxib peak does not interfere with any degrading peak and method is found specific. Reproducibility[9] of method was checked by performing method precision in which same test preparation was performed six times and each of the preparations was injected in duplicate. Results were calculated against average area of five replicate injections of standard preparation. Reproducibility of method was checked by Robustness[10] of method was checked by changing mobile phase composition, column oven temperature and flow rate and found method is robust in all condition and there is no merging of any peaks and peak shapes is also found good. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. Stability of analytical solution was checked and found that it is stable up to 21 hrs at room temperature. For that freshly prepared solution injected and same solution kept at room temperature and injected after 21 hrs. Area difference found within acceptance criteria.

CONCLUSION:

The elution of Celecoxib standard was carried out on Universal C18, 4.6 × 100 mm x 5-micron column HPLC column, at the flow rate of 1.3 ml/min in isocratic mode using the mobile phase consisting of Water: Methanol: Orthrophosphoric acid (38:62:0.1) with flow rate of 1.3 ml/min at 250 nm.

The precision and intermediate precision of the method was calculated in terms of the relative standard deviation, and it was observed very less which indicates accurate reproducibility of method. Linearity graph plotted against average area Vs concentration which is found linear with correlation coefficient 0.9997

This method can be used successfully for identification and quantification of the active pharmaceutical ingredient Celecoxib from pharmaceutical ingredients. Hence this method can be used for the routine analysis of Celecoxib.

performing intermediates precision, in which same experiment was performed by different workers, different days etc. From the data obtained it is concluded that our method is reproducible. This is depicted in table 2 for comparative results.

Table: 2 Precision

Sr.No	MP	IP
1	99.9%	99.9%
2	100.1%	99.8%
3	100.0%	99.6%
4	100.1%	99.4%
5	100.1%	99.8%
6	99.8%	99.5%
Average	99.8%	

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