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A validated LC method for the quantitative determination of celecoxib in pharmaceutical dosage forms and purity evaluation in bulk drugs☆

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Abstract

A new reversed-phase, isocratic LC method was developed for the quantitative determination of COX-2 inhibitor celecoxib in bulk drugs and in pharmaceutical dosages. The proposed method is also applicable for the purity evaluation of celecoxib in bulk drugs. 5-Methyl 2-Nitro phenol has been used as internal standard for the quantitative determination of celecoxib. The method has been completely validated and proven to be rugged. The limit of detection (LOD) and limit of quantitation (LOQ) for celecoxib impurities namely, 4-hydrazino benzene sulfonamide (Intermediate I) and 1-(4-methyl phenyl)-4,4,4-trifluro butan-1,3-dione (Intermediate II) were found to be 32.0 and 97 ng, respectively. The active pharmaceutical ingredient was extracted from its finished dosage form (capsule) using methanol. The percentage recoveries ranged from 90.7 to 93.8. The stability studies were performed for celecoxib solution placed on laboratory bench and in refrigerator for hundred days. The samples were found to be stable for the study period.

Keywords: Celecoxib; Intermediate I; Intermediate II; LOD; LOQ

1. Introduction

Celecoxib (Celebrex) is a non steroidal anti-inflammatory drug (NSAID) that blocks the production of postaglandins by inhibiting cycloxygenase type 2 (COX-2), for the treatment of rheumatoid arthritis and osteoarthritis in adults [1].

The NSAIDs exert most of their anti-inflammatory analgesic and antipyretic activities through the inhibition of prostaglandin H synthase or cycloxygenase (COX). COX is the first enzyme in the prostanoid biosynthetic pathway catalysing the conversion of arachidonic acid to prostaglandin H2 as the first step in the synthesis of prostaglandins, prostacyclins and thromboxanes, all of which act as important mediators of both physiological and inflammatory responses. The discovery of a second inducible isoenzyme has enabled the identification of two major isoforms of COX: the constitutive COX isoform, termed COX-1, and the inducible form, or COX-

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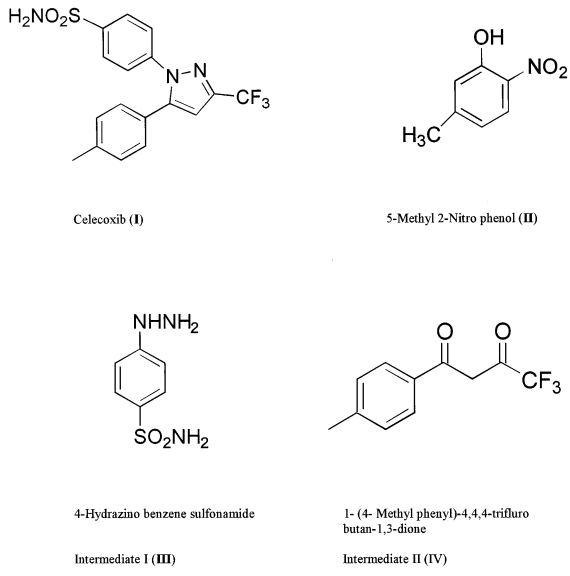

Fig. 1. Chemical structures of celecoxib and its related substances including internal standard.

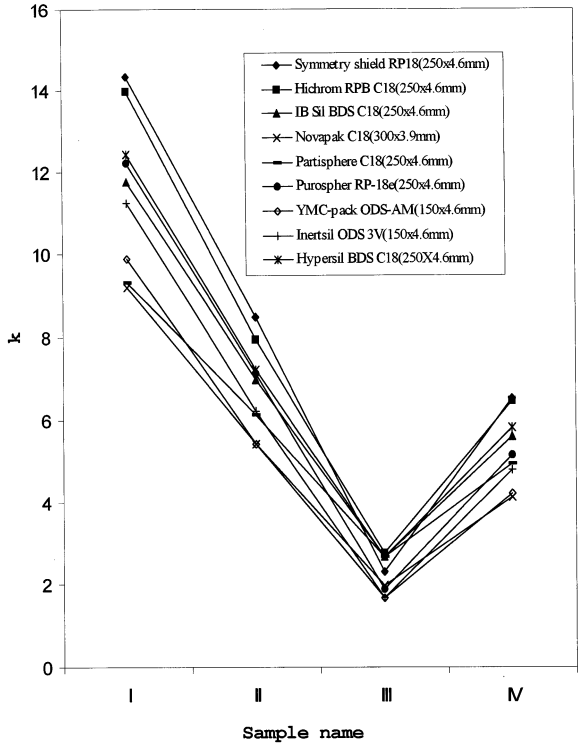

Fig. 2. Method selectivity using different columns.

2. COX-1 is mainly associated with homeostasis, inducible COX-2 would be the major isoenzyme responsible for the production of proinflammatory mediator [2]. It is also observed in the azoxymethane-induced colon carcinogenesis model, celecoxib reduced the incidence, multiplicity and burden of colon cancer by 93, 97 and 89%, respectively [3].

FDA has approved celecoxib recently for marketing the drug worldwide. So far to our present knowledge, no LC methods were reported for the analysis of celecoxib. It is felt necessary to develop a LC method for the purity evaluation and the quantitative determination of celecoxib. The proposed method was tested using nine C18 columns of different make and proven to be versatile, rapid and reliable. 5-Methyl-2-nitro phenol was used as an internal standard for the quantitative determination of celecoxib.

2. Experimental

2.1. Chemicals

Samples of celecoxib, Intermediate I, Intermediate II and 5-methyl 2-nitro phenol were received from Process R&D of Dr Reddy's Research Foundation, Hyderabad, India. Capsules of celebrex (250 mg) were purchased from the market. HPLC grade acetonitrile and methanol were purchased from Merck, USA. Analytical reagent grade potassium dihydrogen phosphate was purchased from S.D. Fine Chemicals Ltd, India. High pure water was prepared by using Millipore Milli Q plus purification system.

2.2. Equipment

The LC system, used in Laboratory A, consisted of a waters 510 solvent delivery system, a Rheodyne injector (7725i) fitted with a 10 μl loop, and a waters 486 tunable absorbance detector. The LC system used in Laboratory B consisted of Perkin–Elmer series 200 lc solvent delivery system, a Rheodyne injector (7725i) fitted with a 10 μl loop and a waters 996 PDA detector. The output signal was monitored and processed using

Table 1
System-suitability report

Compound ($n = 3$)	Capacity factor	Resolution	Tailing factor	No. of theoretical plates
I	8.1	14.4	1.2	15823
II	4.3	7.1	1.4	14048
III	0.8	–	1.9	3154
IV	3.0	13.7	1.3	9371

a millennium 2010 chromatography manager software (Waters) on Pentium computer (Digital Equipment Co.).

2.3. Sample preparation

The stock solutions of celecoxib (2.5 mg/ml) and 5-methyl 2-nitro phenol (1.25 mg/ml) were prepared by dissolving appropriate amounts of the substances in acetonitrile. Celecoxib solutions were prepared from the stock solution by taking 0.1, 0.15, 0.2, 0.25, 0.3 ml, in 5.0-ml volumetric flasks. To maintain uniform concentration (0.25 mg/ml) of internal standard, 1.0 ml of 5-methyl 2-nitro phenol (II) stock solution was added to each celecoxib solution and made up to the mark with acetonitrile.

2.4. Chromatographic conditions

The chromatographic column used was a 300 × 3.9 mm Novapak C18 with 4 μm particles. The mobile phase was aqueous potassium dihydrogen phosphate (pH 4.8; 0.01 M)-acetonitrile (45:55, v/v).The flow rate was 1.0 ml/min. The column was maintained at ambient temperature and the eluant was monitored at a wavelength of 252 nm. The injection volume was 10 μl.

2.5. Chromatographic columns used

The columns used were:

1. Hypersil 5 μm BDS C18 (250 × 4.6 mm), Shandon.
2. IB SIL 5 μm BDS C18 (250 × 4.6 mm), Phenomenex.
3. Hichrom 5 μm RPB (250 × 4.6 mm), Hichrom.
4. Partisphere 5 μm C18 (250 × 4.6 mm), Whatmann.
5. Novapak 4 μm C18 (300 × 3.9 mm), Waters.
6. YMC-Pack 5 μm ODS-AM (150 × 4.6 mm), YMC.

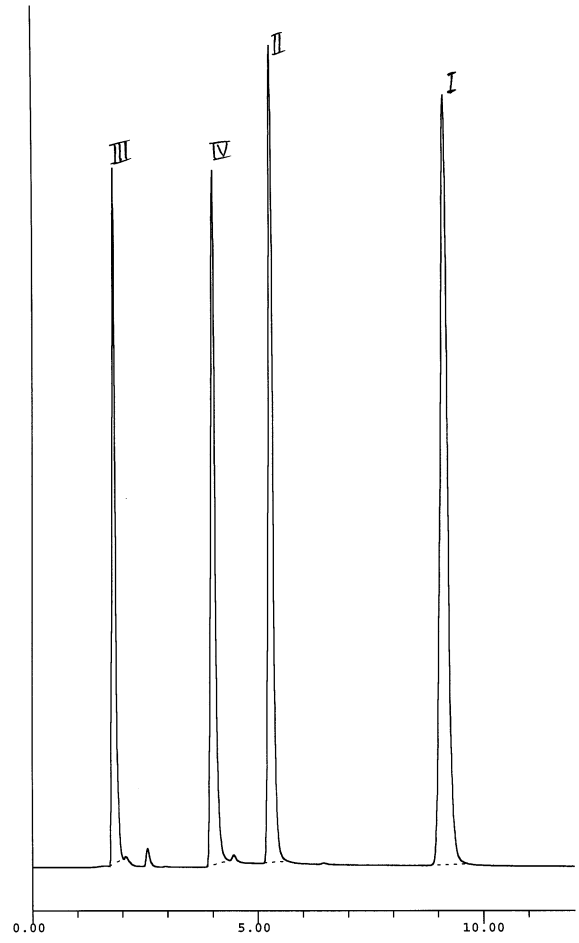

Fig. 3. HPLC chromatogram of celecoxib (0.3 mg/ml) spiked with internal standard and its related substances.

Table 2
Specificity results of the method

	Pure sample	Sample spiking with all the impurities
	101.2	99.3
	101.4	98.8
	100.1	98.5
Mean	100.9	98.8
SD	0.717	0.373
% RSD	0.7	0.3

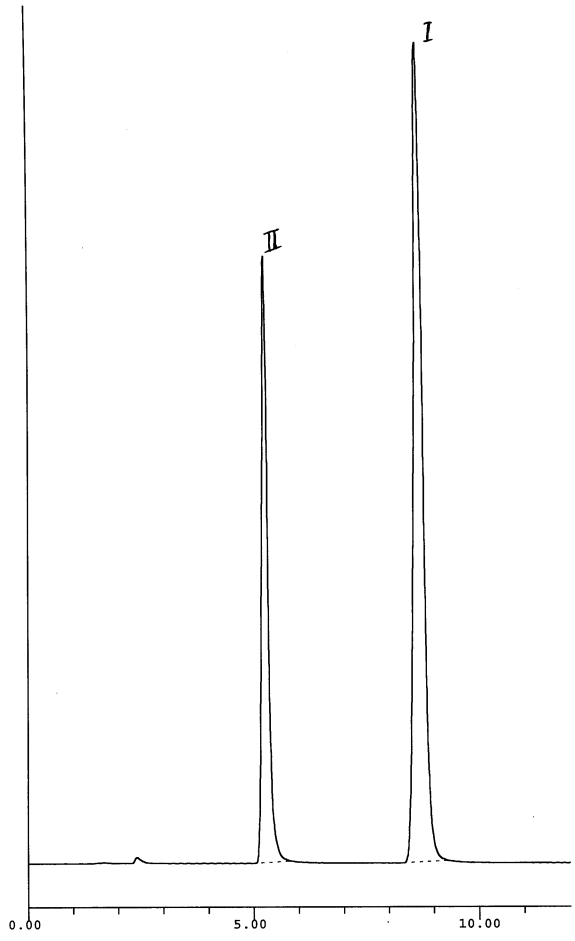

Fig. 4. HPLC chromatogram of formulated celecoxib (celebrex 250 mg) with internal standard (0.15 mg/ml).

7. Purospher 5 μm RP-18e (250 × 4.0 mm), Merck.
8. Inertsil 5 μm ODS 3V (150 × 4.6 mm), G.L.Sciences.
9. Symmetry shield RP-18 5 μm (250 × 4.6 mm), Waters.

Table 3
Intra-and inter-day assay variations of celecoxib

Intra-day			
0 day			
Mean of concentration (mg/ml) $n = 3$	0.0814	0.1195	0.1440
SD	0.0003	0.0003	0.0002
% RSD	0.4	0.3	0.1
1 day			
Mean of concentration (mg/ml) $n = 3$	0.0817	0.1202	0.1475
SD	0.0009	0.0011	0.0008
% RSD	1.1	0.9	0.5
2 day			
Mean of concentration (mg/ml) $n = 3$	0.0821	0.1191	0.1496
SD	0.0012	0.0008	0.0013
% RSD	1.5	0.7	0.9
Inter-day			
Mean of concentration (mg/ml) $n = 3$	0.0817	0.1191	0.1470
SD	0.0003	0.0005	0.0028
% RSD	0.4	0.4	1.9

Table 4
Accuracy in the assay determination of celecoxib

Day of analysis	Taken (mg)	Recovery (mg) $n = 3$	% Recovery[a]
0	0.0804	0.0814	101.2
	0.1191	0.1195	100.3
	0.1438	0.1440	100.1
1	0.0821	0.0817	99.5
	0.1196	0.1202	100.5
	0.1499	0.1475	98.4
2	0.0834	0.0821	98.4
	0.1194	0.1191	99.7
	0.1493	0.1496	100.2

[a] % Recovery = % recovery of celecoxib from the sample against taken.

Table 5
Assay results of formulations of celecoxib

S.	NO	Concentration of celecoxib (mg/ml)		% Assay of celecoxib	% RSD
		Taken	Recovered		
I	1	0.0802	0.0745	93.0	
	2	0.0802	0.0729	91.0	
	3	0.0802	0.0727	90.7	1.3
II	1	0.1018	0.0951	93.4	
	2	0.1018	0.0955	93.9	
	3	0.1018	0.0955	93.9	0.2
III	1	0.1440	0.1324	92.0	
	2	0.1440	0.1319	91.6	
	3	0.1440	0.1324	92.0	0.2

3. Results and discussion

3.1. Method development

To develop a rugged and suitable LC method for the quantitative determination of celecoxib (I) different mobile phases and stationary phases were employed. For purity determination of celecoxib in bulk drug and estimation of impurities present namely Intermediate I (III), Intermediate II (IV), were carried at using proposed chromatographic conditions. The chemical structure of I, II, III and IV are shown in Fig. 1.

In all the columns stated above, the resolution between celecoxib and I.S. was found to be not less than 10 and all the known impurities were eluted in about 20 min (Fig. 2). The peaks are sharp and symmetric in all the columns used. In Novapak C18, 4 μm (300 × 3.9 mm) all the impurities were eluted in about 12 minutes with good resolution. For the method validation Novapak C18, 4 μm (300 × 3.9 mm) was employed. The system suitability results were given in Table 1. The impurities of celecoxib III and IV were identified by their retention times by injecting them separately. The retention times of I, II, III and IV were 9.1, 5.3, 2.0, 4.0 min, respectively (Fig. 3).

3.2. Method validation

3.2.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of all the potential impurities.

For the specificity determination, all the known impurities were added to pure celecoxib sample and the response of the analyte in the mixture was

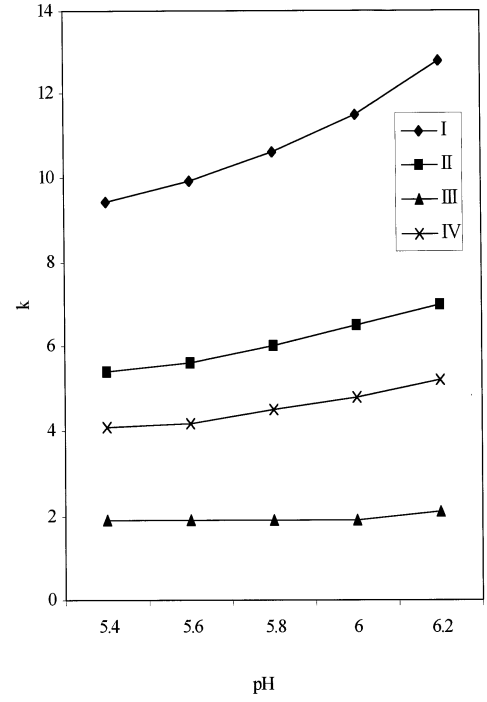

Fig. 5. Effect of change in buffer pH on retention.

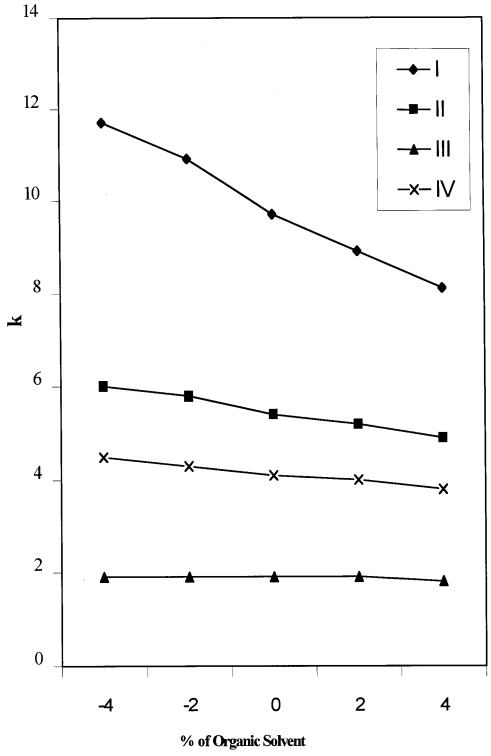

Fig. 6. Effect of change in percentage solvent strength on retention.

compared with the response of the pure celecoxib. It is found that assay results were not changed in the presence of impurities. The assay results were given in the Table 2. The specificity was checked by stressing the pure celexcoxib sample under UV light (254 nm) and temperature 70°C for 24 h and under some extreme conditions such as 0.1 N HCl, 0.1 N NaOH, and 3% H_2O_2 solutions. All the degraded products formed were well separated from celecoxib. In the formulation samples of celecoxib it was noticed that excepient peaks did not interfere with the peaks of interest (Fig. 4). Hence the method is applicable for the quantitative determination of celecoxib in pharmaceutical dosage forms.

Table 7
Recovery of celecoxib impurities

Compound	Added (μg) ($n = 3$)	Recovered (μg)	% Recovery
III	0.2630	0.2622	99.6
	0.5258	0.5087	96.7
	1.0432	1.0453	100.2
IV	0.2552	0.2751	107.7
	0.5443	0.5321	97.7
	1.1126	1.1895	107

3.2.2. Linearity

The calibration curve for celecoxib was drawn by plotting the peak area ratio of celecoxib/I.S. versus concentration of celecoxib yielded coefficient of regression (r^2) 0.9994 over the concentration range of 0.05–0.15 mg/ml. The target analyte concentration of celecoxib was taken as 0.1 mg/

Table 6
Assay results of stability studies of celecoxib solution

Day	Concentration of celecoxib (mg/ml) placed on bench top		% Recovery	% RSD[a]	Concentration of celecoxib (mg/ml) placed in refrigerator		% Recovery	% RSD[a]
	Recovered	Taken			Recovered	Taken		
0	0.1441	0.1438	100.2		0.1442	0.1438	100.3	
10	0.1449	0.1438	100.7		0.1429	0.1438	99.4	
20	0.145	0.1438	100.8		0.1436	0.1438	99.9	
30	0.1444	0.1438	100.4		0.1431	0.1438	99.5	
40	0.1451	0.1438	100.9	0.78	0.1451	0.1438	100.9	0.71
50	0.1444	0.1438	100.4		0.1441	0.1438	100.2	
60	0.1461	0.1438	101.6		0.1442	0.1438	100.3	
80	0.1433	0.1438	99.6		0.1452	0.1438	100.9	
100	0.1422	0.1438	98.9		0.1421	0.1438	98.8	

[a] % RSD calculated for nine determinations.

ml. The regression equation for celecoxib was $y = 15.00x + 0.034$. Linearity was checked for 3 consecutive days for the same concentration range from the same stock solutions. The average slope value of celecoxib was 15.0084 ± 0.042. The RSD values of the slope and intercept for the linearity study were 1.1 and 5.9%, respectively.

3.2.3. Precision and accuracy

Intra day precision and accuracy of the method were evaluated by assaying freshly prepared solutions in triplicate at concentrations of 0.08, 0.12, and 0.14 mg/ml of celecoxib. The RSD ranged from 0.1 to 1.5% (Table 3). Inter day precision and accuracy of the method calculated from the individual recovery data were evaluated by assaying freshly prepared solutions, in triplicate, for 3 days. The RSD ranged from 0.4 to 1.9% for celecoxib (Table 3). The accuracy results in terms of percentage recoveries were shown in Table 4.

3.2.4. Assay of celecoxib in formulation sample

Twenty capsules of celecoxib (celebrex 250 mg, Pfizer) were finely ground using agate mortar and pestle. About 52.93 mg of the ground material, which is equivalent to 40 mg of the active pharmaceutical ingredient (API), was extracted into methanol in a 100-ml volumetric flask by vortex mixing followed by ultrasonication. The resultant mixture was filtered through 0.45-μm membrane filter. The filtrate was used as a stock solution for preparing test solution. Triplicate test solutions were prepared each contain up 1 ml each of stock solution and internal standard in 5 ml of acetonitrile. This solution would correspond to the API concentration of 0.0802 mg/ml. Similar experiments were carried out by weighing 67.18 mg (equivalent to 51 mg of the API) and 95.03 mg (equivalent to 72 mg of the API) to prepare two more test solutions which would contain API at concentrations of 0.1018 and 0.144 mg/ml solutions, respectively. Experiments were performed and the results tabulated in Table 5.

3.2.5. Limit of detection and limit of quantitation

The limit of detection (LOD) represents the concentration of analyte that would yield a signal-to-noise ratio of 3 [4]. LOD for III and IV was 32 ng/ml for 10 μl injection volume.

The limit of quantitation (LOQ) represents the concentration of analyte that would yield a signal-to-noise ratio of 10 [4]. LOQ for III and IV was 97 ng/ml for 10 μl injection volume.

3.2.6. Ruggedness

The ruggedness of an assay method is defined as degree of reproducibility of assay results obtained by analysis of the same sample under variety of normal test conditions such as different labs, different analysts, different instruments, and different lots of reagents. The same samples of three concentration levels in triplicate of day 3 were analysed at laboratory B with different instrument (Perkin-Elmer series 200 lc solvent delivery system, a Rheodyne injector (7725i) fitted with a 10 μl loop and a waters 996 PDA detector) by different analyst. The data obtained from the laboratory B is well in agreement with the data of day 3 results obtained in laboratory A.

3.2.7. Robustness

The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, percentage organic solvent strength, and buffer concentration. To determine robustness of the method experimental conditions were purposely altered and chromatographic characteristics were evaluated.

The total pH of the mobile phase was 5.8. To study the pH effect on the retention (k) of celecoxib, I.S., and two potential impurities of celecoxib, buffer pH was changed by 0.2 units from 5.4 to 6.2. Increase in retention (k) with increase in pH was noticed. The results were shown in Fig. 5. The effect of temperature on the retention characteristic (k) of celecoxib, I.S., and two impurities of celecoxib was studied by changing the temperature in steps of 2°C from 24 to 32°C. Variation in temperature did not have a significant effect on resolution and peak shape. Effect of percent organic strength on retention was studied by varying the percentage of acetonitrile from -4 to $+4$% while the other mobile phase components were held constant as stated in section 2.4. Slight decrease in k was observed with increase in the levels of acetonitrile. The results were shown

in Fig. 6. Effect of buffer concentration was checked at four concentration levels, i.e. 0.01, 0.025, 0.05, and 0.1 M. No significant change was observed in retention and peak shape.

3.2.8. Stability

The stability of 0.14 mg/ml solutions of celecoxib was evaluated. The solutions were stored in a tightly capped volumetric flask, on a laboratory bench and in the refrigerator. Recovery of these solutions was checked for a hundred days against freshly prepared solutions. The samples kept in the refrigerator and on the laboratory bench were found to be stable (Table 6).

3.2.9. Standard addition and recovery of impurities

Standard addition and recovery experiments were conducted to determine the accuracy of the present method, for the quantification of impurities. The range of addition levels of impurities is 0.05–2.5 wt% of target analyte concentration [5]. The range of addition level of impurities used in this study is 0.05–1.0 wt% and the recovery of each impurity is calculated from the slope and the intercept of the calibration curve drawn in the concentration range of 0.125–2.5 μg/ml. The response factors for impurities III and IV were found to be 0.55 and 0.67, respectively.

The equations for the calibration curve for III and IV were $y = 21451x - 342$ and $y = 22959x + 937$, respectively. The RSD values of the slope and the intercept for the calibration equation of III and IV were 1.4, 2.6 and 2.1 and 4.3%, respectively. The percentage recoveries of impurities ranged from 96.7 to 107.7 (Table 7).

4. Conclusions

An isocratic reversed-phase LC method has been described for the quantitative determination of celecoxib. The method is also applicable for the purity evaluation of celecoxib. The method was extensively validated and it was found to be rugged and robust.

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