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LC separation of *ortho* and *meta* isomers of celecoxib in bulk and formulations using a chiral column

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Abstract

A normal phase, isocratic LC method was developed for the separation of positional isomers of celecoxib (I) using a chiral column, Chiralpak-AD. The method is useful for the quantification of *ortho* (II) and *meta* (III) forms in bulk drugs and formulation samples of celecoxib. The method has been completely validated and proven to be rugged. The limit of detection (LOD) and limit of quantitation (LOQ) of *ortho* and *meta* forms were found to be 38 ng and 116 ng respectively. The active pharmaceutical ingredient was extracted from its finished dosage form (capsule) using ethanol. The percentage recoveries of *ortho* isomer was found to be 99.8–102.7 and 97.8–103.2 and the percentage recoveries of *meta* isomer was found to be 99.3–102.6 and 99.7–104 in spiked bulk and formulation samples of celecoxib respectively.

Keywords: Celecoxib; *Ortho* form; *Meta* form; LOD; LOQ; Capsule

1. Introduction

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

The compound was designed to reduce the adverse effects associated with conventional nonsteriodal anti-inflammatory agents [2]. Celecoxib showed excellent selectivity for COX-2 over COX-1 in in-vitro studies. In a number of in-vivo models, celecoxib demonstrated potent anti-inflammatory activity after oral dosing. It decreased acute inflammation in the carragenan-induced edema and reduced chronic inflammation in the adjuvant arthritis model. These results were similar to that obtained with standard NSAIDs, but in contrast to the latter, celecoxib produced neither acute nor chronic gastrointestinal toxicity [3].

To our knowledge, there was no LC method reported for the separation of positional isomers of celecoxib till date with chiral or achiral column in open literature. In the preparation of celecoxib 1-(4-methyl phenyl)-4,4,4-trifluro butan-1,3-dione was one of the key intermediates. The key intermediate was prepared from 4-methyl acetophe-

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none. 4-methyl acetophenone was in turn prepared from toluene by acetylation using a Friedal Craft's reagent. During this reaction acetylation at *ortho* and *meta* position leads to the formation of 2-methyl and 3-methyl acetophenones. These contaminants were carried over all the way to final and result in the formation of *ortho* and *meta* isomers as impurities. The schematic diagram was presented in Fig. 1. The authors have published a reversed phase LC method about the purity evaluation and assay determination of celecoxib in bulk and pharmaceutical samples in the previous paper [4]. This method suffered from resolution between *ortho* and *meta* isomers of celecoxib. Hence it was felt necessary to develop a LC method for the separation of positional isomers of celecoxib (*ortho*, *meta* and *para*). All our efforts to separate these two impurities from main product celecoxib using normal and reverse phase HPLC, under varied conditions were not successful. It has been reported in the literature for the separation of *meta* and *para* isomers of fexofenadine using a chiral column [5]. Taking this as clue, a successful attempt was made for the separation and quantification of positional isomers of celecoxib using a Chiralpak AD column in bulk and formulation samples. The method was extensively validated and it was found to be selective and rugged.

2. Experimental

2.1. Chemicals

Samples of celecoxib, *ortho* and *meta* isomers of celecoxib were received from Process Research Group of Dr. Reddy's Research Foundation, Hyderabad, India. Capsules of celebrex (250 mg) were purchased from Pfizer, USA. HPLC grade Hexane was purchased from s.d.-fine chemicals, India and ethanol was purchased from Zhuhai Hongtu Industries Corp., China.

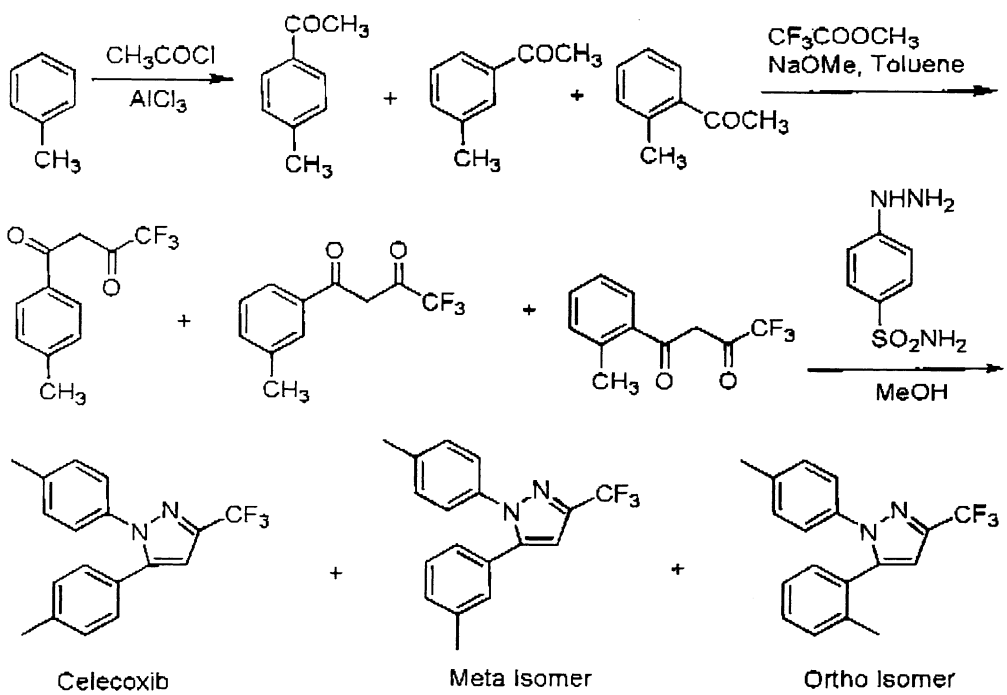

Fig. 1. A schematic diagram of formation of celecoxib and its isomers.

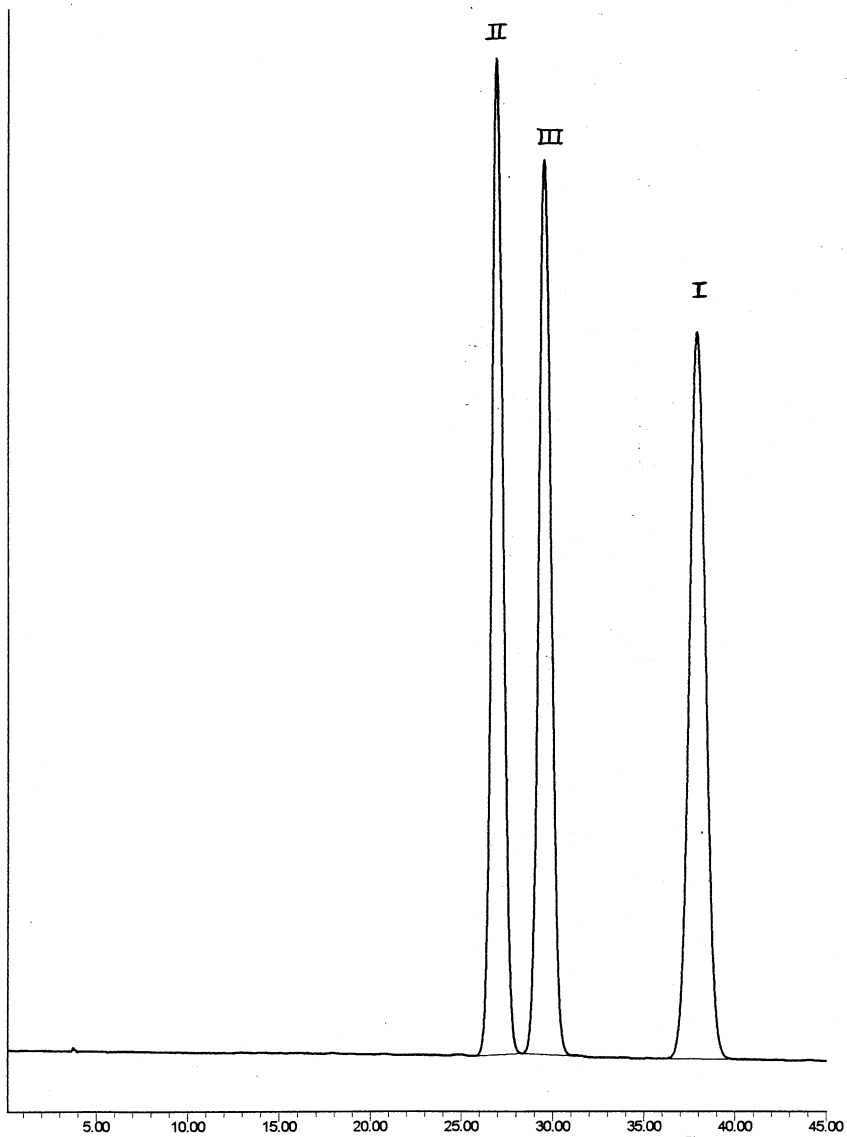

Fig. 2. HPLC Chromatogram of celecoxib and its isomers (0.5 mg/ml).

Table 1
System-suitability report

Compound ($n = 3$)	Capacity factor	Resolution	Tailing factor	No. of theoretical plates
I Celecoxib	36.2	–	1.3	6215
II *Ortho* isomer	24.9	1.92	1.2	6567
III *Meta* isomer	28.2	4.93	1.1	7254

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

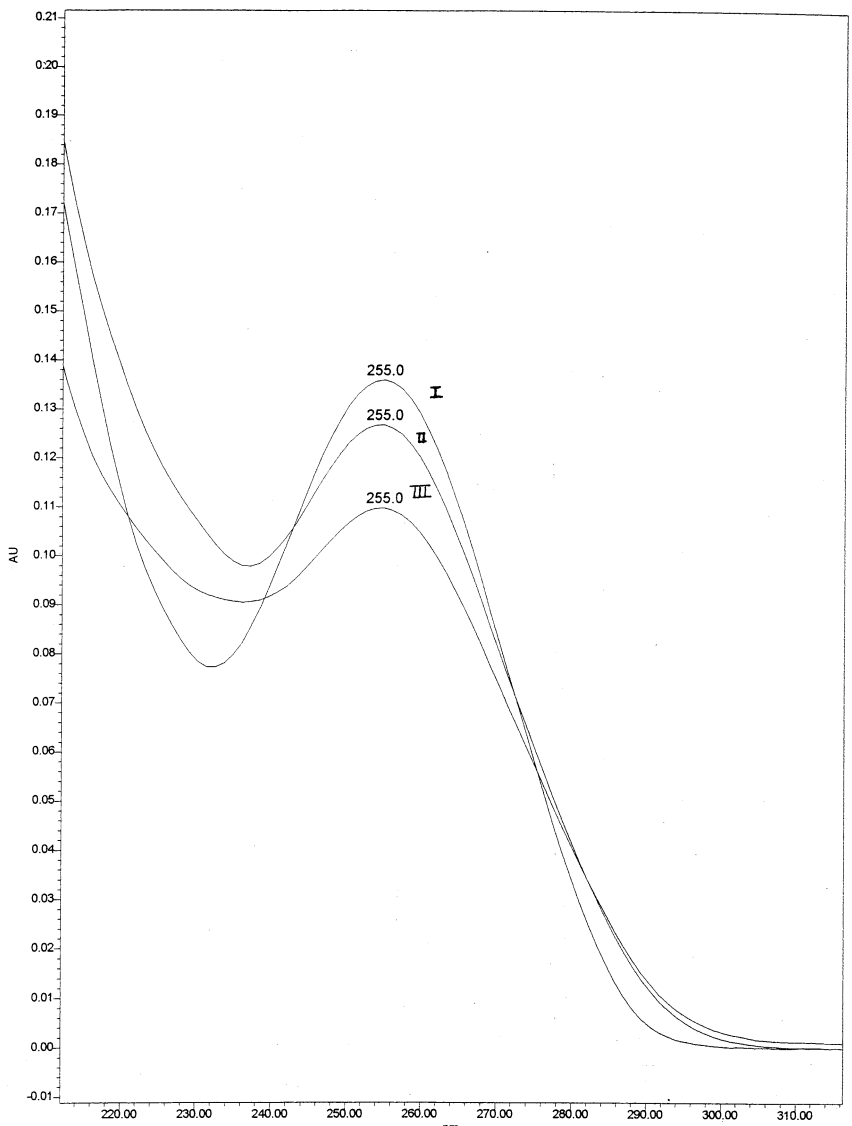

Fig. 3. UV spectra of celecoxib and its isomers.

Table 2
Recovery of impurities in bulk drug samples in Laboratory A

Compound	Added (μg) ($n = 3$)	Recovered (μg)	% Recovery	% RSD
II	0.6911	0.6992	101.1	1.3
	1.6656	1.6625	99.8	0.7
	4.42	4.539	102.7	0.2
III	0.6418	0.6585	102.6	0.9
	1.8129	1.7997	99.3	1.1
	4.631	4.71	101.7	0.5

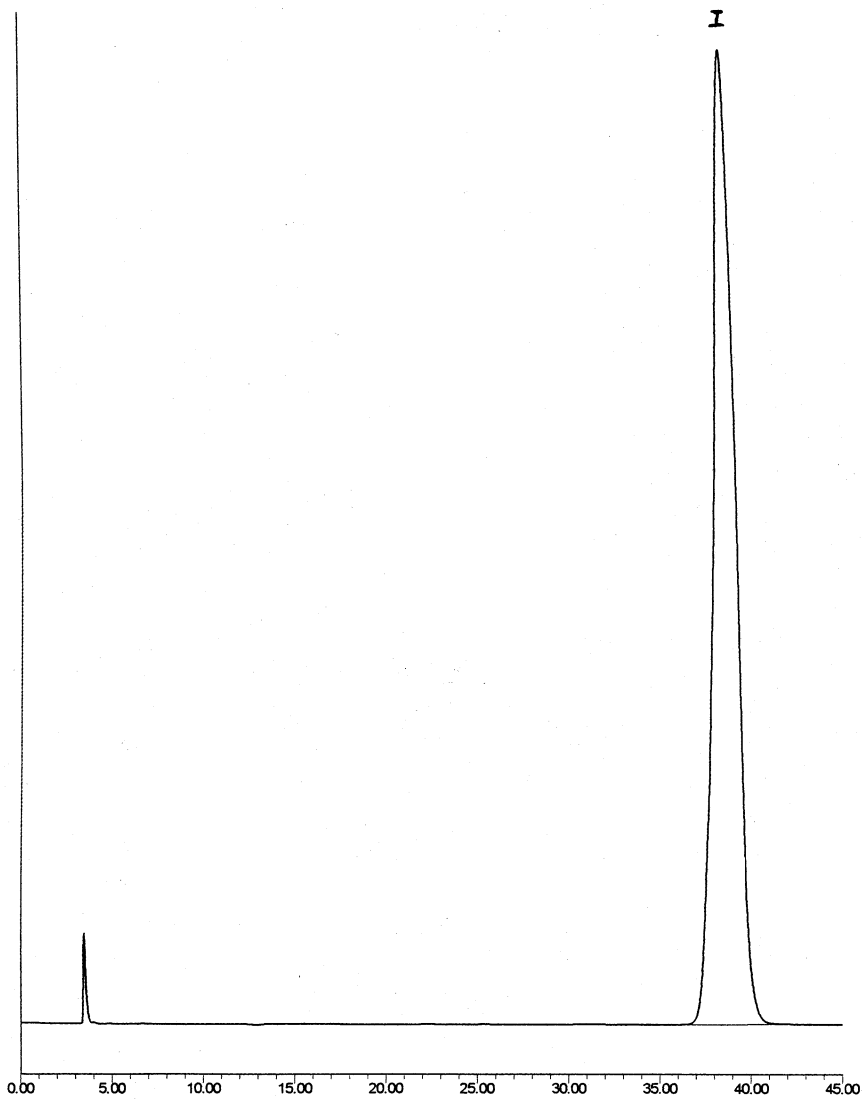

Fig. 4. HPLC chromatogram of formulated celecoxib (0.5 mg/ml).

2.3. Sample preparation

The stock solutions of celecoxib and its *ortho* and *meta* isomers (2.5 mg/ml) were prepared by dissolving appropriate amounts of substances in ethanol.

2.4. Chromatographic conditions

The chromatographic column used was a 250 × 4.6 mm Chiralpak AD with 10 μm particles which was accompanied with a 5 cm long guard column. The mobile phase was hexane: ethanol (94:06,

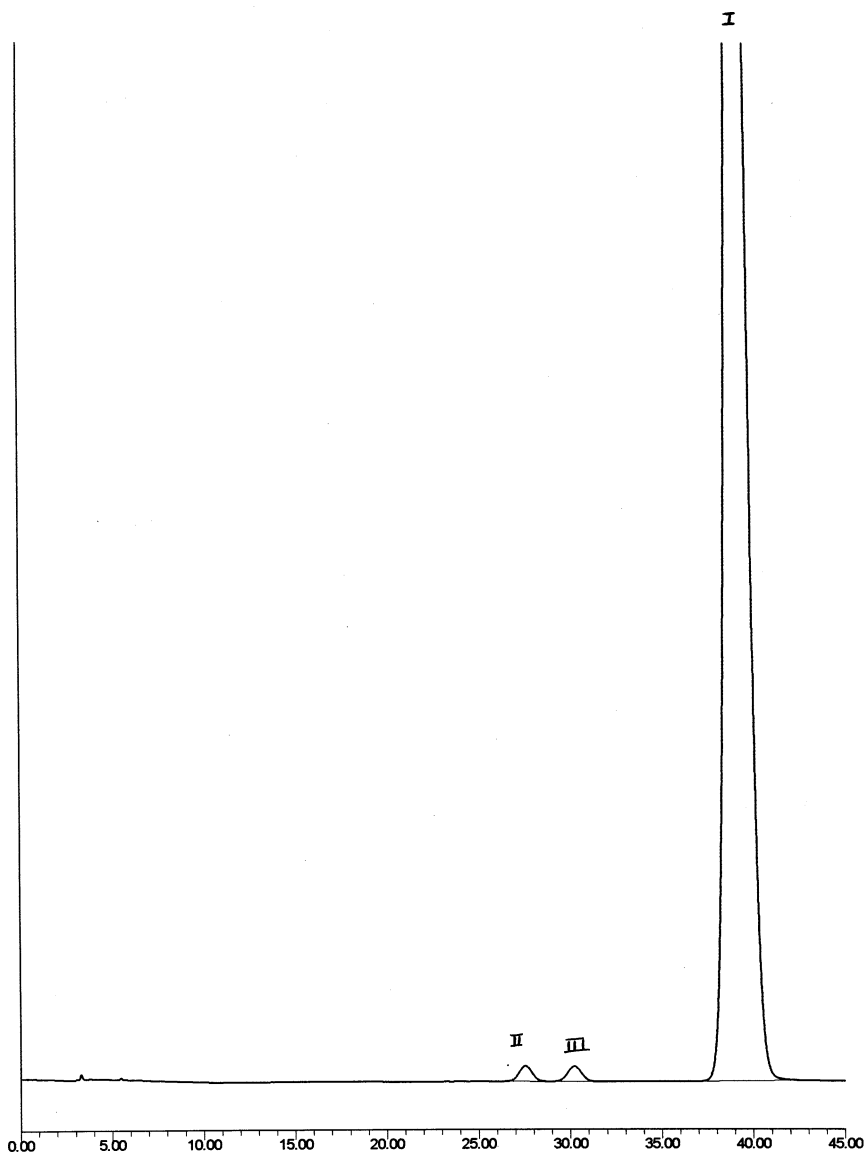

Fig. 5. HPLC chromatogram of the formulated sample blended with *ortho* and *meta* isomers (0.9% w/w).

Table 3
Recovery of impurities in formulation samples in Laboratory A

Compound	Added (μg) ($n = 3$)	Recovered (μg)	% Recovery	% RSD
II	0.8303	0.8128	97.8	1.3
	1.6682	1.6383	98.2	0.2
	4.4372	4.5802	103.2	1.7
III	0.9115	0.9093	99.7	0.9
	1.8334	1.8472	100.7	1.2
	4.6466	4.8289	104	1.4

v/v). The flow rate of the mobile phase was 1.0 ml/min. The column was maintained at ambient temperature and the eluant was monitored at a wavelength of 255 nm. The injection volume was 10 μl.

3. Results and discussion

3.1. Method development

The aim of this work was for the separation and quantification of *ortho* and *meta* isomers of celecoxib in bulk and formulation samples of celecoxib. To develop a rugged and suitable LC method for the separation of positional isomers of celecoxib, different mobile phases and stationary phases were employed. In the preliminary trials, separation was attempted using reversed phase columns such as C18 and C8 of different make and by changing different mobile phase conditions. But in all these trials *ortho* and *meta* isomers could not be separated. In an attempt to separate *ortho* and *meta* isomers, various chiral columns such as Chiralcel OD-R (Daicel) and Cyclobond I 2000 AC column (Astec) were employed by using various possible mobile phases. Separation was not achieved in all these trails. In Chiralcel OD column of Daicel, surprisingly all the isomers of celecoxib were coeluted. In Chiralpak AD column, *ortho* and *meta* isomers of celecoxib could be separated with good resolution using a mobile phase system consisting of hexane: ethanol (94:06 v/v). Chiralpak AD column was an amylose based chiral column. Amylose was a well-known polysaccharide; the monomer of amylose was D-glucose, which was the same as that of cellulose. However, amyloses said to have helix structure based on the α-linkage of D-glucose units [6]. All the isomers of celecoxib were also separated by using iso-propanol as an organic modifier. But in the system consisting of ethanol, the resolution was better compared to iso-propanol system. In the above-cited conditions celecoxib and its isomers eluted below 45 min and they were identified by retention times by injecting the reference standards separately. The retention times of *ortho*, *meta* isomers and celecoxib were 26, 29 and 37 min respectively (Fig. 2). The system suitability results were given in Table 1.

3.2. Quantification of impurities in bulk drug

Celecoxib (bulk) sample provided by process research group of Dr. Reddy's Research Foundation did not show presence of *ortho* and *meta* isomers. Pure *ortho* and *meta* isomers were studied for their UV response and relative response factors (RRF). The absorption maxima for all the

Table 4
Accuracy in the assay determination of celecoxib

Taken (mg)	Recovery (mg)	% Recovery [a]	% RSD
0.4273	0.4250	99.5	
0.4273	0.4353	101.9	1.3
0.4273	0.4344	101.7	
0.5984	0.5952	99.4	
0.5984	0.5995	100.2	0.6
0.5984	0.6023	100.7	
0.5638	0.5634	99.9	
0.5638	0.5615	99.7	0.9
0.5638	0.5534	98.2	

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

Table 5
Ruggedness data of impurities in laboratory B

Compound ($n = 3$)	Concentration of impurities in bulk drug samples (μg/ml)		% Recovery	Concentration of impurities in formulation samples (μg/ml)		% Recovery
	Added	Recovered		Added	Recovered	
I	0.6911	0.7112	103	0.8303	0.8119	97.7
	1.6656	1.6423	98.6	1.6682	1.6899	101.3
	4.42	4.562	103.2	4.4372	4.6525	104.8
III	0.6418	0.6325	98.5	0.9115	0.8964	98.3
	1.8129	1.8596	102.5	1.8334	1.8546	101.1
	4.631	4.343	93.7	4.6466	4.9521	106.5

Table 6
Assay results of stability studies of celecoxib and its isomers on laboratory bench

Compound	Day	Added (μg) ($n = 3$)	Recovered (μg)	% Recovery	% RSD[a]
II	1	5.2114	5.2029	99.8	
	2	5.2114	5.3132	101.9	
	3	5.2114	5.1985	99.7	1.0
	4	5.2114	5.2103	99.9	
	5	5.2114	5.1436	98.7	
III	1	5.1035	5.0116	98.2	
	2	5.1035	5.0323	98.6	
	3	5.1035	5.0728	99.4	0.9
	4	5.1035	5.1188	100.3	
	5	5.1035	5.1392	100.7	
I	1	525.2	521.4	99.3	
	2	525.2	518.1	98.6	
	3	525.2	531.8	101.2	1.1
	4	525.2	526.7	100.2	
	5	525.2	524.2	99.8	

[a] % RSD calculated for five determinations.

compounds were found to be 255 nm. RRFs were calculated by injecting the mixture of celecoxib and its isomers at unit concentration (0.5 mg/ml). The absorption spectrum of the isomers of celecoxib was shown in Fig. 3. The UV detection was carried out at 255 nm for the quantification of impurities of celecoxib (*o*, *m* isomers of celecoxib). RRFs have been used for the quantification of impurities [7]. The RRFs of *ortho* and *meta* forms were found to be 0.96 and 0.93 respectively. Standard addition and recovery experiments were conducted to determine accuracy of the present method for the quantification of impurities. The target analyte concentration of celecoxib was chosen as 0.5 mg/ml. As per ICH guidelines, the quantification of impurities is to be studied in the range of 0.05–2.5%, w/w of target analyte concentration [8]. The study was carried out at 0.125, 0.3 and 0.9 weight percent of target analyte concentration. The recovery of each impurity was calculated from the slope and the intercept of the calibration curve drawn in the concentration of 0.5–7.5 μg/ml (0.1–1.5% w/w). The calibration equations for *ortho* and *meta* isomers were $y = 15893x - 813$ and $y = 16201x - 552$ respectively. The RSD values of the slope and the intercept for the calibration equations of *ortho* and *meta* isomers were 1.3, 3.5 and 3.4, 5.4% respectively. The

percentage recoveries of impurities ranged from 99.8 to 102.7 (Table 2).

3.3. Quantification of impurities in formulation samples

One capsule of celebrex (250 mg, Pfizer) was finely ground using agate mortar and pestle. The ground material which was equivalent to 125 mg of the active pharmaceutical ingredient (API), was extracted into ethanol in a 50-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. 10 ml of this solution was taken in a 50 ml volumetric flask and made up to the volume with ethanol. This solution corresponds to a concentration of 0.5 mg/ml.

Interestingly there were no *ortho* and *meta* isomers present in the celecoxib formulation sample (Fig. 4). The *ortho* and *meta* isomers were added to the extracted 0.5 mg/ml formulation samples in the same range as described and the recoveries were calculated as under Section 3.2. The chromatogram of the formulation blended with *ortho* and *meta* isomers was shown in Fig. 5. The percentage recoveries of *ortho* and *meta* isomers were ranged from 97.8 to 104 respectively (Table 3).

3.4. Linearity and accuracy of parent compound

The method was also useful to determine linearity and accuracy of the parent compound.The calibration curve for celecoxib was drawn by plotting the peak area of celecoxib versus concentration of celecoxib yielded a coefficient of regression 0.9999 over the concentration range of 0.25–0.75 mg/ml. The regression line equation for celecoxib was $y = 30554394x + 25188$. The RSD value of the slope and the intercept for the calibration equation was 1.5 and 3.3% respectively. The accuracy of the method was evaluated by assaying freshly prepared solutions in triplicate at concentrations of 0.4, 0.55 and 0.60 mg/ml. The percentage recoveries ranged from 98.2 to 101.9 (Table 4).

3.5. 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 [9]. LOD for II and III was 38 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 [9]. LOQ for II and III was 116 ng/ml for 10 μl injection volume.

Table 7
Assay results of stability studies of celecoxib and its isomers in refrigerator

Compound	Day	Added (μg) ($n = 3$)	Recovered (μg)	% Recovery	% RSD[a]
II	1	5.2114	5.2189	100.1	
	2	5.2114	5.3121	101.9	
	3	5.2114	5.2374	100.5	1.5
	4	5.2114	5.1436	98.7	
	5	5.2114	5.1175	98.2	
III	1	5.1035	5.0473	98.9	
	2	5.1035	5.1256	100.4	
	3	5.1035	5.0321	98.6	1.1
	4	5.1035	5. 1391	100.7	
	5	5.1035	5.1478	100.9	
I	1	525.2	531.2	101.1	
	2	525.2	524.9	99.9	
	3	525.2	516.8	98.4	1.0
	4	525.2	527.3	100.4	
	5	525.2	528.9	100.7	

[a] % RSD calculated for five determinations.

3.6. Ruggedness

The ruggedness of an assay method was 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 standard addition and recovery experiments of the impurities at the same concentration levels used in laboratory A were done at laboratory B with different instrument by different analyst. The data obtained from the laboratory B is well in agreement with the results obtained in laboratory A (Table 5).

3.7. Stability

Solution stability of celecoxib in presence of its positional isomers (*ortho* and *meta*) was studied. Celecoxib (5.0 mg/ml) and isomer solutions of 10.0% w/w were prepared in ethanol. 1.0 ml of each of these solutions were taken in a 10.0 ml volumetric flask in duplicate and made up to the volume with mobile phase. The mixture constitutes a concentration of 1.0% w/w isomers. The stability of the mixture in the refrigerator and on the laboratory bench was studied. Recovery of these solutions was checked for a period of 5 days against freshly prepared solutions. The stability data of the samples kept on the laboratory bench and in the refrigerator are shown in Tables 6 and 7. No degradation was observed and they are found to be stable.

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References

[1] Daily Drug News.com (Daily Essentials) January 4, 1999.
[2] Drug Data Rep 19(2) (1997) 161.
[3] A. Grail, A.M. Martel, J. Casstaner, Drugs Fut. 22 (7) (1997) 711.
[4] M.K. Srinivasu, Ch. Lakshmi Narayana, D. Sreenivas Rao, G. Om Reddy, Journal of Pharmaceutical and Biomedical Analysis 22 (2000) 949–956.
[5] US Pharmacopeial Forum; volume 25, number 23, May–June 1999, pp. 8050–8054.
[6] R. Ferritti, B. Gallinella, F. La Torre, L. Turchetto, Journal of Chromatography A 769 (1997) 231–238.
[7] M.P. Newton, John Mascho, Randy J. Maddux, in: Satinder Ahuja (Ed.), Chromatography of Pharmaceuticals, American Chemical Society, Washington, DC, 1992, pp. 41–53.
[8] J. Mark Green, Analytical Chemistry News and Features May 1, 1996, 305A–309A.
[9] International conference on Harmonization, Draft Guideline on Validation Procedures: Definitions and Terminology, Federal Register, Volume 60, pp. 11260, March 1, 1995.