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SPECTROFLUORIMETRIC DETERMINATION OF CELECOXIB IN BULK AND FORMULATIONS

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ABSTRACT

A simple, rapid and economical spectrofluorimetric method for estimation of Celecoxib, a selective COX-2 inhibitor, in bulk and solid dosage forms was developed in the present study. Celecoxib shows maximum excitation intensity at 245 nm and maximum emission intensity at 382 nm which is used in this study. The linear regression equation obtained by least square regression method, was Int =0.253*Conc. (in ng/ml) +34.80. The method provides a linear response across a quantization range of 250ng/ml to 4000ng/ml. The developed method was employed with a high degree of precision and accuracy for the estimation of total drug content in three commercial capsule formulations of Celecoxib. The results of analysis were treated statistically, as per International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures. The results were found to be accurate, reproducible and free from interference.

Keywords: Celecoxib; Spectrofluorimeter; Recovery Studies

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INTRODUCTION

Celecoxib (Figure 1) chemically (4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzene sulfonamide is a COX-2 inhibitor used for treatment of osteoarthritis and rheumatoid arthritis. Cyclooxygenase (COX) catalyzes the first step in the biosynthesis of prostanoids, the generation of prostaglandinH2 from arachidonic acid. Two forms of COX exist. The constitutive form (COX-1) is found in healthy tissues and produces physiologically important prostaglandins while the inducible form (COX-2) is predominantly expressed during inflammatory conditions[1,2]. Conventional nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both forms of COX and inhibit platelet aggregation[3]. Due to Celecoxib's specificity for the COX-2 inhibition, it has the potential to cause less gastropathy and risk of GI bleeding[3,4]. Celecoxib has also been indicated for its chemo preventive activity in case of colon carcinogenesis[5], UV light induced skin cancer[6] and breast cancer[7].

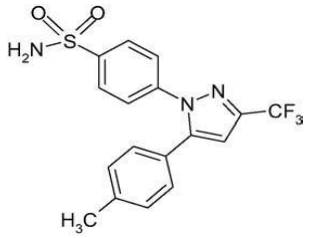
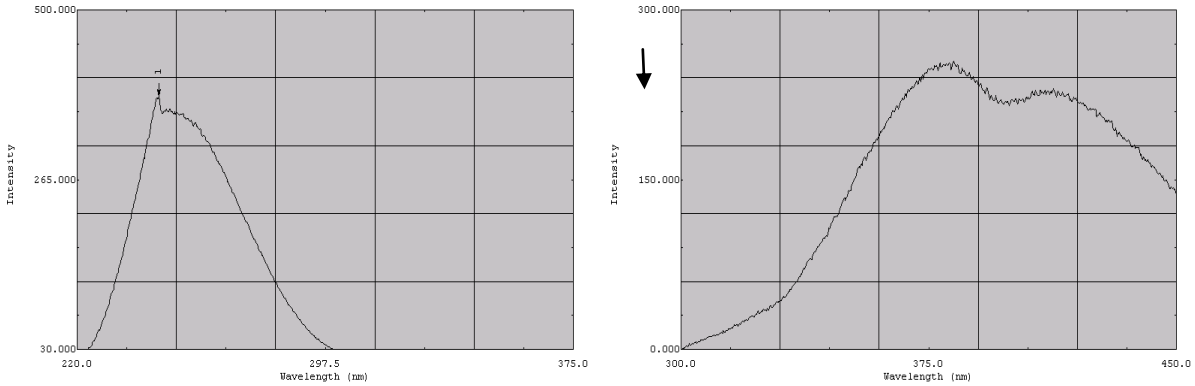


Figure 1. Structural formula of Celecoxib.

As the use of Celecoxib is increasing , many analytical methods like micellar electro kinetic chromatography (MEKC)[8], spectrophotometry[9], first-derivative spectrophotometry[10],thin-layer chromatography (TLC)[10], adsorptive stripping voltammetry[11], liquid chromatography (LC)[12,13] liquid chromatography–mass spectrometry(LC–MS)[14–16], solid-phase extraction and high-performance liquid chromatography (SPE-HPLC)[17], high-performance liquid chromatography with UV[18-21] and fluorimetric[21] detection have been described for the determination of Celecoxib in pharmaceutical formulation and human plasma. In the present study, our intention was to develop a simple spectrofluorimetric method for determination of Celecoxib in bulk and solid dosage forms. Spectrofluorimetric method has advantages like high sensitivity, selectivity, easy to operate, economic and could be easily adapted for routine quality control analysis, dissolution or similar studies. In this method Celecoxib showed excitation wavelength at 245 nm and emission wavelength at 382nm as shown in Figure 2, the developed method was used to estimate the total drug content in three commercially available capsules of Celecoxib. The results of the analysis were validated by statistical methods[22,23] and recovery studies.



(I) (II)

Figure 2: Excitation Spectrum (I) and Emission Spectrum (II) of Celecoxib

MATERIALS AND METHODS

Apparatus

Fluorescence spectra and intensity measurements were made on a Shimadzu RF-5301 PC spectrofluorimeter equipped with single quartz cell of 1 cm path length.

Reagents

Pure Celecoxib was obtained from Aarthi Drugs Limited (India). Methanol (Analytical Grade) was purchased from S.D. Fine Chemicals Ltd., Mumbai, India. Three commercially available capsules of Celecoxib were selected from the local market on a random basis. These capsules normally contain common additives like diluents, glidants and lubricants (magnesium stearate, etc.). All solutions were prepared in triple distilled water. The materials and vessels used for analysis were precleaned by soaking in sulfochromic acid mixture (saturated $K_2Cr_2O_7$in concentrated H_2SO_4) for at least 1 h and subsequently rinsed four times with triple distilled water before use.

Method development

Solvent Selection:To develop a rugged and suitable spectrofluorimetric method for the analysis of Celecoxib in formulations, different solvents systems such as water, methanol, ethanol, acetonitrile, 0.1N HCl, 0.1N NaOH and different combinations of these were used. The criteria employed for assessing the suitability of a particular solvent system for the drug was cost, time required for analysis, sensitivity of the assay, use of the same solvent system for extraction of the drug from the formulation excipient matrix for estimation of the drug content. Finally methanol was selected as suitable solvent system.

Preparation of standard stock solution: A 100 µg/ml stock standard solution of Celecoxib was prepared by dissolving 10.0 mg of drug in little quantity of methanol and diluting it to the mark in a 100 ml volumetric flask with methanol.

Selection of excitation and emission λmax :The standard stock solution was scanned between 220 and 375 nm for excitation wavelength keeping 400 nm as emission wavelength using same solvent system for auto zero. From the spectrum obtained, 245 nm was selected as excitation λmax for the analysis of celecoxib, then keeping the excitation wave length as 245 nm the stock solution was scanned was scanned between 300nm-450nm for emission wavelength using same solvent system as blank. From the spectrum obtained, 382 nm was selected as emission λmax for the analysis of celecoxib.

Calibration graph:From the standard stock solution, various dilutions were made to obtain solutions of 250, 500, 1000, 2000, 3000, 4000ng/ml with the same solvent system just before use and fluorescence intensity was measured for each dilution at 245nm as excitation wavelength and 382 nm as emission wavelength. Five sets of Calibration graph standards were analyzed to avoid variation. The calibration curve was plotted between concentration and average intensity in the concentration range from 250 to 4000 ng/mL. The results are listed in Table 1.

Method validation

Accuracy: The accuracy of the method was checked by recovery determinations. The determination was done over three concentration levels in triplicate according to the ICH guidelines[22]. The concentration levels selected as quantifiable concentration samples were 500ng/ml, 1500ng/ml and 3500ng/ml.

Precision: The precision was evaluated on the basis of repeatability and intermediate precision. On at least three occasions, three replicates of each quantifiable concentration sample pool at low, middle, and high concentrations were assayed according to the ICH requirements[22]. Percentage relative standard deviation (%RSD) was calculated for both the methods.

Linearity: Five separate series of solutions of the drug, in the concentration range 250-4000ng/ml were prepared from the stock solution and analyzed.

Limit of detection (LOD) and quantification (LOQ): LOD and LOQ were calculated on the basis of response and slope of the regression equation.

Robustness and Ruggedness: The robustness was determined by preparing stock solution in 9:1 Methanol water system, while the ruggedness of the method was established by changing the analyst and the percent recovery was determined. The results obtained were subjected to

unpaired t-test with actual percent recovery values to find any significant difference.

Estimation of Celecoxib from three commercial capsule formulations by the proposed method:

Three commercially available capsules of Celecoxib (Brand A, B and C) were taken randomly from the Indian market for estimation of total drug content per capsule by the proposed method. For each brand, 20 capsules were weighed, contents were thoroughly mixed and an accurately weighed aliquot amount (equivalent to 10 mg of Celecoxib) was dissolved in about 20 ml methanol. The resulting solution was filtered through Whatman filter paper no. 1 into a 100 ml volumetric flask. The residue was washed several times with methanol and solution was diluted to the mark with the same solvent. The resulting solutions were suitably diluted to get final concentration within the limits of linearity for the respective proposed method (as given in Table 6). From the fluorescence intensity value the drug content per capsule (on an average weight basis) was calculated. The results are tabulated in Table 6.

RESULTS AND DISCUSSION

Method development

The spectra of celecoxib were shown in Figure 2 , excitation and emission wavelengths of celecoxib were at 245 and 382 nm respectively in the selected solvent system. The statistical analysis of data obtained for the estimation of celecoxib in pure solution indicated a high level of precision for the proposed method as evidenced by low standard deviation values (Table1). The low values of coefficient of variation (Table 1) further established the precision of the proposed method. The linear regression equation obtained was $Y= 0.253.X+34.80$ ($r^2= 0.996$), where Y is the Fluorescence intensity and X is the concentration (in ng/ml) of pure celecoxib solution. The correlation coefficient value obtained were highly significant for the method (Table 2).

Table 1 Calibration curve points of the proposed method for estimation of Celecoxib

Concentration of the solution (ng/ml)	Mean intensity value*	Coefficient of variation (%)
250	82.70±1.70	2.06
500	159.45±6.20	3.89
1000	283.24±8.21	2.90
2000	566.11±10.54	1.86
3000	822.41±11.38	1.38
4000	1015.90±2.56	0.25

*Average of five determinations with standard deviation.

A one-way ANOVA test [24, 25] was performed based on the values observed for each pure drug concentration during the replicate measurement of the standard solutions. The calculated *F*-value (F_{Calc}) was found to be less than the critical *F*-value (F_{Crit}) at 95% significance levels in this method (Table 3).

Table 2 Results of least square regression analysis of data for the estimation of Celecoxib by the proposed method

Statistical parameters	Value
Re egression equation [a]	Y = 0.253X + 34.80
Correlation coefficient (r^2)	0.996
Standard error of slope	7.35×10^{-4}
Standard error of intercept on ordinate	1.1110

[a]Based on five calibration values.

Table 3 One-way ANOVA test for linearity of pure Celecoxib solution by the proposed method

Source of variation	Degree of freedom(DF)	Sum of squares(SS)	Mean sum of squares(MS)	F-value	
				F_{calc}	F_{crit}*
Between group	4	25.60	6.401	0.000045	2.73
Within group	25	3555000	142200		
Total	29	3555000			

* Theoretical value of *F* (4, 25) based on one-way ANOVA test at *P*=0.05 level of significance

Validation of the developed method

The developed method was validated according to the standard procedures [10,11] and the results obtained are tabulated in Table 4 and 5. The linearity range of celecoxib solution was found to be 250 – 4000 ng/ml. The accuracy of these estimations was calculated on the basis of percent recovery and results varied between 98.99 – 101.759%. Repeatability and intermediate precisions calculated on the basis of percentage relative standard deviation on replicate set of calibration samples (n = 3 for each concentration) was less than 5%. For the developed method, varying the concentration of methanol from 100% to 90% in stock solution did not significantly (α=0.05) affect the sensitivity by applying unpaired t-test which indicates that method was robust. The proposed method was found to be rugged when analyst was varied. The limit of detection (LOD) and limit of quantitation (LOQ) were given in Table 5. From these values we can say that the proposed method for estimation of celecoxib has excellent sensitivity .

Recovery studies

The method was evaluated by estimation of Celecoxib in pharmaceutical formulations by the proposed method and analysis of pure drug solution as reference. The results are presented in Table 6. The percentage recovery from these formulations by the proposed method varied from

95.64% to 97.37% method. The estimated drug content with low values of standard deviation established the precision of the proposed methods. Common formulation excipients in the concentration normally used did not affect the fluorescence intensity of the drug.

Table 4 Results of Validation parameters

Analytical parameter	500 ng/ml	1500ng/ml	3500ng/ml
Accuracy (%RECOVERY)	101.759	100.706	98.994
Repeatability (%RSD)	2.165	1.283	1.182
Intermediate precision (%RSD)	1.998	1.550	0.721
Ruggedness (t_{cal})	0.33	1.86	0.30
Robustness (t_{cal})	1.59	1.61	2.17

t_{tab} value at 95% confidence interval, d.f = 4, two-sided is 2.78

Table 5 Results of other Validation parameters

Analytical parameter	Value
Linearity	250-4000ng/ml
Limit of detection (LOD	32.42ng/ml
Limit of quantitation (LOQ)	98.26ng/ml

Table 6 Results of the assay of pure celecoxib and commercial formulations by the proposed method

Sample	Label claim (mg/capsule)	Results	
		AR (%)	CV%[a]
Pure drug solution[b]		98.46	0.24
Brand A	100	96.87	2.65
Brand B	100	95.64	2.90
Brand C	100	97.37	1.78

CV, coefficient of variation; AR, analytical recovery.
a CV for triplicate determinations.
b 10 mg in 100 ml.

CONCLUSION

The method was suitable for determination of Celecoxib in bulk samples and solid dosage forms. The method described was simple, reproducible and sensitive with enough accuracy and precision. In addition it was rapid and more economical than other methods reported in the literature. It is possible to quantify concentrations down to 98.26 ng/ml with detection limit of 32.42g/ml. The sample recoveries in all formulations were in good agreement with their respective label claims and thus suggested non-interference of formulation excipients. This method was better when compared to other reported methods and thus can be used effectively, for routine analysis of Celecoxib in bulk form and its formulations and can also be used for dissolution or similar studies.

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REFERENCES

1. Vane JR, Botting RM. New insights into the mode of action of anti-inflammatory drugs. Inflamm Res 1995; 44:1-10.
2. Crofford LJ. COX-1 and COX-2 tissue expression: implications and predictions. J Rheumatol 1997; 24: Suppl 49: 9-15.
3. Goldenberg MM. Celecoxib, a selective cyclooxygenase-2 inhibitor for the treatment of rheumatoid arthritis and osteoarthritis. Clin Ther. 1999; 21: 1497–1513.
4. Fort J. celecoxib, aCOX-2 specific inhibitor : the clinical data. Am J orthop. 1999; 28 suppl:13 – 18.
5. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res. 1998; 58: 409–412.
6. Fisher SM, Lo HH, Gordon GB, Seibert K, Kellof G, Lubet RA, Conti CJ. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. Mol Carcinog. 1999;25(4): 231–240.
7. Harris RE, Alshafie GA, Abou-Issa H, Seibert K. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. Cancer Res. 2000; 60 (8):2101–2103.
8. Srinivasu MS, Rao DS, Reddy GO. Determination of celecoxib, a COX-2 inhibitor, in pharmaceutical dosage forms by MEKC. J Pharm Biomed Anal 2002; 28: 493–500.
9. Saha RN, Sajeev C, Jadhav PR, Patil SP, Srinivasan N. Determination of celecoxib in pharmaceutical formulations using UV spectrophotometry and liquid chromatography. J Pharm Biomed Anal 2002; 28: 741–751.
10. Bebawy LI, Mostafa AA, AboTalib NF. Stability-indicating methods for the determination of doxazosin mezylate and Celecoxib. J Pharm Biomed Anal 2002; 27:779–793.
11. Ghoneim MM, Beltagi AM. Adsorptive stripping voltammetric determination of the anti-inflammatory drug celecoxib in pharmaceutical formulation and human serum. Talanta 2003; 60:911–921.

12. Srinivasu MK, Narayana CL, Rao DS, Reddy GO. A validated LC method for the quantitative determination of celecoxib in pharmaceutical dosage forms and purity evaluation in bulk drugs. J Pharm Biomed Anal 2000; 22: 949–956.
13. Rao DS, Srinivasu MK, Narayana CL, Reddy GO. LC separation of ortho and Meta isomers of celecoxib in bulk and formulations using a chiral column. J Pharm Biomed Anal 2001; 25: 21-30.
14. Abdel-Hamid M, Novotny L, Hamaz H. Liquid chromatographic mass spectrometric determination of celecoxib in plasma using single-ion monitoring and its use in clinical pharmacokinetics. J Chromatogr B 2001; 753: 401–408.
15. Brautigam L,Vetter G, Tegeder I, Heinkele G, Geisslinger G. Determination of celecoxib in human plasma and rat microdialysis samples by liquid chromatography tandem mass spectrometry. J Chromatogr B 2001; 761:203–212.
16. Werner U, Werner D, Mundkowski R, Gillich M, Brune K. Selective and rapid liquid chromatography–mass spectrometry method for the quantification of rofecoxib in pharmacokinetic studies with humans. J Chromatogr B 2001; 760:83–90.
17. Chow HHS, Anavy N, Salazar D, Frank DH, Alberts DS. Determination of celecoxib in human plasma using solid-phase extraction and high-performance liquid chromatography. J Pharm Biomed Anal 2004; 34:167–174.
18. Jalalzadeh H, Amini M, Ziaee V, Safa A, Farsam H, Shafiee A. Determination of celecoxib in human plasma by high-performance liquid chromatography. J Pharm Biomed Anal 2004; 35: 665–670.
19. Rose MJ, Woolf EJ, Matuszewski BK. Determination of celecoxib in human plasma by normal-phase high-performance liquid chromatography with column switching and ultraviolet absorbance detection. J Chromatogr B 2000; 738: 377–385.
20. Stormer E, Bauer S, Kirchheiner J, Brockmoller J, Roots I. Simultaneous determination of celecoxib, hydroxycelecoxib, and carboxycelecoxib in human plasma using gradient reversed-phase liquid chromatography with ultraviolet absorbance detection. J Chromatogr B 2003; 783: 207–212.
21. Schonberger F, Heinkele G, Murdter TE, Brenner S, Klotz U, Hofmann U. Simple and sensitive method for the determination of Celecoxib in human serum by high-performance liquid chromatography with fluorescence detection. J Chromatogr B 2002; 768: 255–260.

22. International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Harmonised Tripartite Guideline on Validation of Analytical Procedures: Methodology, Recommended for Adoption at Step 4 of the ICH Process on November 1996 by the ICH Steering Committee, IFPMA,Switzerland.
23. United States Pharmacoepia, United States Pharmacoepial Convention, Inc., 24th ed., Rockville, USA, 2000: 2149 – 2152.
24. Duncan R, Knapp RG, Miller MC, Introductory Biostatistics for the Health Sciences, 2nd ed., Delmer Publishers Inc, 1983:115 – 150.
25. Bolton S. In: Pharmaceutical Statistics: Practical and Clinical Application, 3rd ed., Marcel Dekker, New York, 1997; 153: 216 – 269.