Available online at www.sciencedirect.com
SCIENCE DIRECT®
IL FARMACO 59 (2004) 139–145
IL FARMACO
www.elsevier.com/locate/farmac

Derivative spectrophotometric and fluorimetric methods for determination of rofecoxib in tablets and in human plasma in presence of its photo-degradation product

Mostafa A. Shehata *, Nagiba Y. Hassan, Ahmad S. Fayed, Badr A. El-Zeany

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasrel Aini Street, Cairo 11562, Egypt

Received 16 July 2003; accepted 8 November 2003

Abstract

Rofecoxib (I) has been determined in the presence of its photo-degradation product (II) using first derivative spectrophotometry (^{1}D) and first derivative of the ratio spectra (^{1}DD) by measuring the amplitude at 316.3 and 284 nm for ^{1}D and ^{1}DD, respectively. (I) can be determined in the presence of up to 70% and 80% of (II) by the ^{1}D and ^{1}DD, respectively. The linearity range of both the methods was the same (5.8–26.2 μg ml^{-1}) with mean percentage recovery of 100.08 ± 0.84 and 100.06 ± 1.06 for ^{1}D and ^{1}DD, respectively. ^{1}D method was used to study kinetics of (I) photo-degradation that was found to follow a first-order reaction. The $t^{1/2}$ was 20.2 min while K (reaction rate constant) was 0.0336 mol min^{-1}. Both methods were applied to the analysis of (I) in bulk powder and in pharmaceutical formulations. Also a spectrofluorimetric method is described to determine (I) at very low concentrations (25–540 ng ml^{-1}) where (I) is converted to its photo-degradate (II), which possesses a native fluorescence that could be measured. The proposed method was applied for the analysis of tablets containing rofecoxib as well as to rofecoxib-spiked human plasma.

Keywords: Rofecoxib; Derivative spectrophotometry; Kinetics; Fluorimetry

1. Introduction

Many synthetic non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting both cyclo-oxygenase enzymes namely (cox-1 and cox-2); cox-1 predominates in the stomach yielding protective prostaglandins while cox-2 is induced during inflammation giving rise to pain, swelling and stiffness. The use of these NSAIDs such as aspirin or ibuprofen may cause gastric ulcers and hemorrhagia upon chronic use [1]. Due to selective inhibition of prostaglandin synthesis, cox inhibitors are most useful as gastric safe NSAIDs that were developed in the last few years. Of these drugs, rofecoxib has been proved to be highly selective cox-2 inhibitor with a selectivity ratio of more than 800 [2]. Few methods have been reported for determination of rofecoxib, which are mainly liquid chromatographic ones [3]. Determination of rofecoxib in plasma by HPLC methods has also been described [4–6]. Solid-phase extraction has also been used for determination of (I) in plasma [7]. An HPLC method has also been reported for determination of (I) in the presence of related process intermediate and other related compounds [8]. LC–MS methods have been developed for quantification of (I) in bioavailability and pharmacokinetic studies [9,10]. Structure determination of rofecoxib from powder diffraction data using molecular packing analysis has been reported [11]. The aim of this work is to develop sensitive stability indicating spectrophotometric and spectrofluorimetric methods for the determination of rofecoxib in the presence of its photo-degradation product. The developed methods were applied for the analysis of rofecoxib in some pharmaceuticals and in human plasma. A kinetic study of the photo-degradation reaction was also carried out.

2. Experimental

2.1. Instrumentation

- A double-beam UV–visible spectrophotometer (SHIMADZU, Japan) Model UV-1601 PC connected to IBM

* Corresponding author.
E-mail address: mostafa1960@yahoo.com (M.A. Shehata).

doi:10.1016/j.farmac.2003.11.010

compatible computer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2 nm and wavelength-scanning speed was 2800 nm min^{-1}.
- A spectrofluorimeter (SHIMADZU, Japan) Model RF-1501 with an Epson Lx-300+ printer. One centimeter quartz cells were used at low sensitivity and 2.5 nm bandwidth.
- UV lamp: (4 W lamp) UVP com. Cambridge UK, Model UVGL 25.

2.2. Materials and reagents

- Rofecoxib standard material was kindly supplied from Global Nabi and was certified to have 99.7% purity minimum.
- Vioxx tablet 12.5 mg batch number A/29701, ABR 2994 and 25 mg batch number 29801, 29802 manufactured by Global Nabi Co. under license from Merck & Co., Inc., Whitehouse Station, NJ, USA.
- Acetonitrile HPLC grade (Merck).
- Tablet excepients (placebo): magnesium stearate 1 mg, microcrystalline cellulose 80 mg, lactose monohydrate 8 mg, hydroxy propyl cellulose 6 mg, croscaramelose sodium 8 mg and yellow ferric oxide 0.66 mg were mixed.

2.3. Standard solutions and calibrations for spectrophotometric methods

All glassware used for (I) must be actinic glass or covered with aluminum foil.

2.3.1. Standard solution of rofecoxib

A stock standard solution of rofecoxib was prepared by dissolving 20 mg of accurately weighed rofecoxib in 15 ml acetonitrile in a 25-ml volumetric flask; the volume was completed to mark with acetonitrile. Working standard solution was prepared by diluting 5 ml of stock solution with acetonitrile/water (1:1) in 50-ml volumetric flask so as to obtain a final concentration 80 μg ml^{-1}. The standard solutions were prepared by accurately diluting aliquots of the working solution of (I) with acetonitrile/water (1:1) to obtain the concentration range 5.8–26.2 μg ml^{-1}.

2.3.2. Working standard degradate solutions

Five milliliters of stock standard solution of rofecoxib were transferred into a 100-ml volumetric flask and diluted to mark with acetonitrile/water (1:1). The obtained solution concentration was 40 μg ml^{-1}. The solution was transferred into a quartz cuvette and tightly covered and then subjected to UV lamp (4 W) at 15 cm distance for 45 min. Standard degradate solutions were prepared by diluting aliquots of working standard degradate solution with acetonitrile/water (1:1) to obtain a solution in the concentration range 5–20 μg ml^{-1}.

2.3.3. Calibration for ^1D method

The ^0D absorption spectra for standard solutions of both (I) and (II) were scanned in the range 200–400 nm. ^1D curves were computed at $\Delta\lambda$ = 4 nm and scaling factor 100. The amplitude values of ^1D were measured at 316.3 nm of I (zero crossing of II) and at 273.1 nm for II (zero crossing of I) for determination of (I) and (II), respectively. The concentration was calculated from the regression equations for (I), $A = 0.0905C - 0.0219$ and for (II), $A = 0.3975C + 0.24$ where A is the ^1D amplitude at 316.3 and 273.1 nm for (I) and (II), respectively, and C is the concentration in μg ml^{-1}.

2.3.4. Calibration for ^1DD method

The UV absorption spectra of standard solutions of (I) were divided by the spectrum of 10 μg ml^{-1} solution of (II). The first derivative for the obtained ratio spectra was computed at $\Delta\lambda$ = 8 nm and scaling factor 4. The amplitudes at 284 nm were measured and found in linear relationship with concentration of (I). The concentration was computed from the regression equation, $A = 0.1915C - 0.1161$ where A is the amplitude of ^1DD at 284.0 nm and C is the concentration in μg ml^{-1}.

2.4. Standard solutions and calibration for spectrofluorimetric method

Five milliliters of solution of (I) working solution (40 μg ml^{-1}) were diluted to 50 ml with acetonitrile/water (1:1). A series of standard solutions was prepared in the concentration range 22–550 ng ml^{-1}. Solutions were transferred into covered quartz cell and subjected to irradiation for 5 min then measured fluorimetrically at excitation λ = 247 nm and emission λ = 377 nm. The concentration was computed from the regression equation, $I = 0.993C + 5.418$ where I is the relative fluorescence intensity and C is the concentration in ng ml^{-1}.

2.5. Pharmaceutical formulations

Ten tablets were transferred into 500-ml volumetric flask, 6 ml of water was added and swirled for 10 min. Then 350 ml of acetonitrile was added. The flasks were placed in an ultrasonic bath for 1 h and completed to volume and filtered. Aliquots were transferred to 50-ml flasks and diluted with acetonitrile/water (1:1) so as to obtain concentration 15 μg ml^{-1}. The general procedure under ^1D and ^1DD were followed. For fluorimetry, 1 ml of the solution (15 μg ml^{-1}) was diluted to 100 ml with acetonitrile/water (1:1) and then measured fluorimetrically.

2.6. Application to human plasma

In a series of 10-ml Wassermann tubes, 1 ml blank (drug-free) plasma sample was spiked with different concentrations of rofecoxib to provide final concentrations from 22 to 550 ng ml^{-1}. The samples were vortexed for 2 min, 1 ml of acetonitrile was added to each sample, vortexed for 10 s,

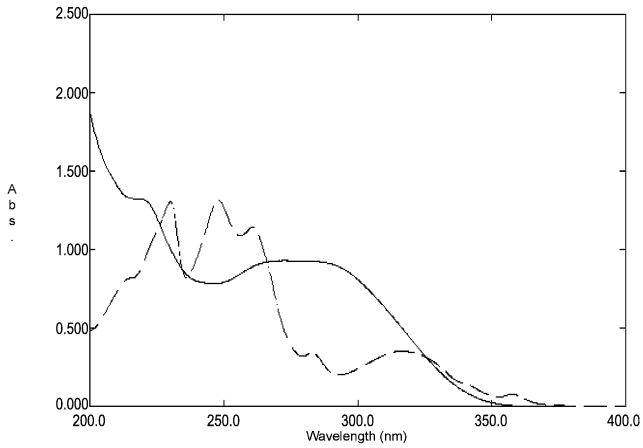
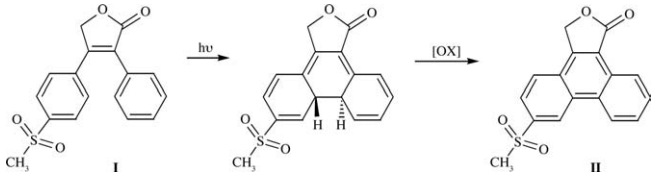


Fig. 1. Zero-order absorption spectra of rofecoxib 23.6 μg ml^{-1} (____) and its photo-degradate 10.3 μg ml^{-1} (_ _ _) in acetonitrile/water (1:1).

Table 1
Results of laboratory prepared mixture of rofecoxib and its photo-degradate by ^{1}D and ^{1}DD methods

Ratio of (I):(II)	Recovery (%) ^{1}D at 316.3 nm for (I)	^{1}DD at 284.0 nm for (I)	^{1}D at 273.1 nm for (II)
100:00	101.10	99.50	
90:10	99.89	97.90	95.50
80:20	100.22	99.30	98.90
70:30	100.51	98.70	99.30
60:40	100.43	98.40	99.30
50:50	97.12	98.80	100.38
40:60	98.45	100.30	97.99
30:70	106.90 [a]	101.00	101.46
20:80	114.30 [a]	104.90 [a]	95.50
10:90		117.90 [a]	99.45
Mean	99.67	99.24	98.64
S.D.	1.395	1.019	2.022

[a] Rejected values.



Scheme 1

centrifuged for 10 min. One milliliter of the upper layer was transferred to 10-ml volumetric flask and completed to volume with acetonitrile/water (1:1) then filtered through a 0.45-μm Millipore filter. The solutions were used for fluorimetric analysis.

2.7. Kinetic studies

Three different concentrations of (I) 0.066, 0.077 and 0.088 × 10^{-3} M were prepared in acetonitrile/water (1:1). Solutions were transferred into quartz cuvette and tightly covered. They were subjected to irradiation with UV lamp (4 W) at a distance of 25 cm. The irradiated solutions were measured by the ^{1}D method at 5-min interval for 30 min.

3. Results and discussion

Rofecoxib is a photo-sensitive drug. The photo-degradation product of (I) has been studied and identified as 6-methyl sulphonyl phenanthro [9,10-C] furan-1 (3H)-one [4]. The degradate is formed through stilbene–phenanthrene like cyclization followed by oxidation. The reaction follows Scheme 1 [4].

The photo-degradate was obtained by exposure of (I) to UV radiation. Trials to prepare standard solutions of pure photo-degradate were carried out by exposure of standard

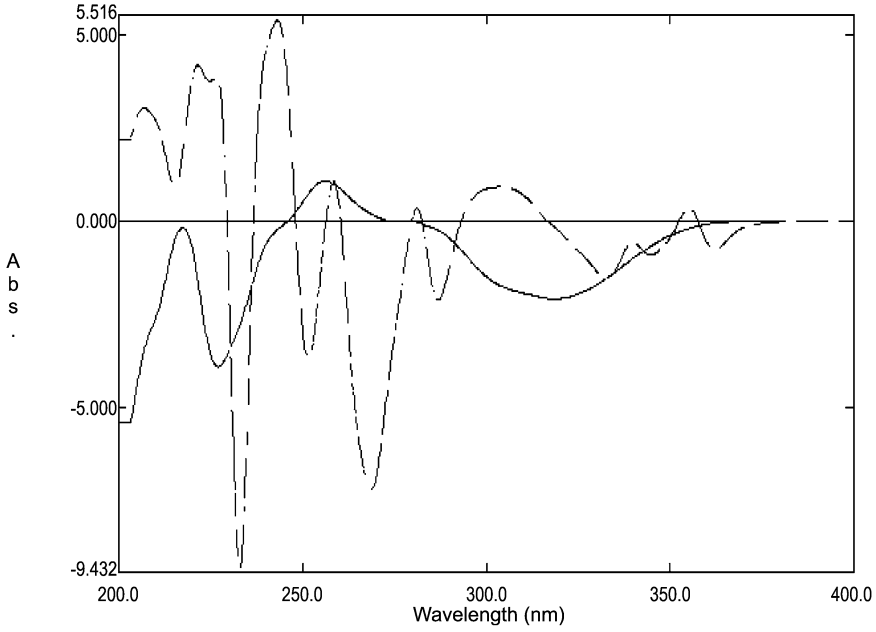


Fig. 2. First derivative curve of the absorption spectra of rofecoxib 23.6 μg ml^{-1} (____) and its photo-degradate 10.3 μg ml^{-1} (_ _ _) in acetonitrile/water (1:1).

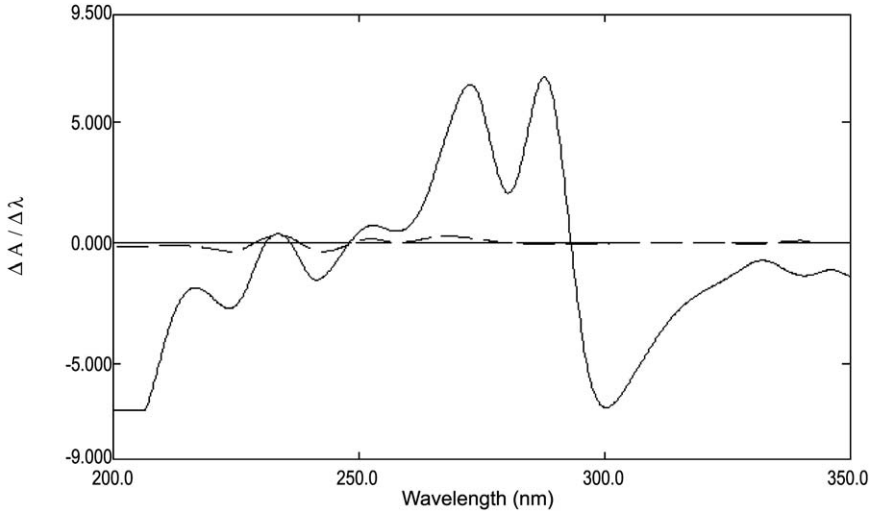


Fig. 3. First derivative curve of the ratio spectra of 23.6 μg ml^{-1} rofecoxib (__) and its photo-degradate 20.6 μg ml^{-1} (_ _ _) using 10.3 μg ml^{-1} degradate as divisor in acetonitrile/water (1:1).

solutions of (I) to UV lamp 254 nm 4 W at a distance of 15 cm. The best conditions for preparation of degradate (II) were by subjecting solutions of (I) in the concentration range 40–50 μg ml^{-1} in acetonitrile/water (1:1) for 45 min. The UV absorption spectrum of each of (I) and (II) typically agreed with those mentioned in literature [3] (Fig. 1). Detection for complete conversion to photo-degradate was confirmed by TLC using cyclohexane/methylene chloride/diethyl amine (50:40:10, v/v) as a solvent system. The R_f values for (I) and (II) were 0.656 and 0.419, respectively.

3.1. ^1D method

The absorption spectra of both (I) and (II) showed severe overlap, hence assay of rofecoxib in presence of its photo-degradate was not successful. ^1D curve on the other hand, as presented in Fig. 2 showed that (I) could be determined in presence of (II) at 316.3 nm at which (II) existed a zero crossing while (II) could be determined at 273.1 nm which is zero crossing of (I). To assess the validity of the method for determination of (I) and (II) in presence of each other, mixtures of (I) and (II) were prepared in ratios shown in Table 1. Using ^1D for determination of (I) at 316.3 nm good recovery was obtained in mixtures containing up to 70% of (II). Meanwhile, (II) could be determined in presence of up to 90% of (I) by measuring the ^1D amplitude at 273.1 nm.

3.2. ^1DD method

The ^1DD (derivative ratio spectra method) was also tried. The main advantage of the method is that the whole spectrum

Table 2
Results of accuracy for ^1D and ^1DD methods

Concentration (μg ml^{-1})	Found (μg ml^{-1})			Recovery (%)		
	^1D at 316.3 nm for (I)	^1DD at 284 nm for (I)	^1D at 273.1 nm for (II)	^1D at 316.3 nm for (I)	^1DD at 284 nm for (I)	^1D at 273.1 nm for (II)
5.82	5.83	5.92		100.17	101.70	
8.84			8.64			97.73
10.24			10.35			101.12
11.67	11.71	11.78		100.4	101.10	
13.23			13.14			99.32
14.72	14.51	14.79		100.00		
14.93			14.05			100.80
17.47	17.40	17.38		99.57	99.50	
17.66	17.64	17.76		99.88	100.56	
17.64			17.61			99.83
20.00	20.41			101.57		
20.61	20.59	20.47		99.89	99.32	
23.29	23.32	23.12		100.12	99.26	
26.2	26.37	26.49		100.65	101.10	
26.46	26.05	26.10		98.45	98.55	
Mean				100.08	100.06	99.76
S.D.				0.840	1.067	1.204
RSD%				0.837	1.05	1.208

Table 3
Results of rofecoxib in tablets, recoveries of added to Placebo by ^{1}D method and ^{1}DD methods

Placebo taken (mg)	Added rofecoxib (mg)	Found (mg)		Recovery (%) [a]	
		^{1}D at 316.3 nm	^{1}DD at 284.0 nm	^{1}D at 316.3 nm	^{1}DD at 284.0 nm
50.1	5.5	5.60	5.56	101.8	101.2
51.2	7.9	8.00	7.93	101.3	100.4
102	10.8	10.72	10.79	99.3	99.9
103.1	16	15.60	15.60	97.5	97.5
102.7	20.6	21.40	21.34	104.0	103.8
Mean				100.78	100.66
S.D.				2.22	2.27
RSD%				2.20	2.25

[a] Mean of two experiments.

Table 4
Results of accuracy by ^{1}D method and ^{1}DD methods

Preparation	Mean found (mg) ± S.D.		Standard addition technique [a] (Recovery (%) ± S.D.)	
	^{1}D at 316.3 nm	^{1}DD at 284.0 nm	^{1}D at 316.3 nm	^{1}DD at 284.0 nm
Vioxx tablet 12.5 mg	11.89 ± 0.077	12.0 ± 0.067	100.65 ± 2.226	99.35 ± 2.73
B.N. A 29701	RSD% = 0.65	RSD% = 0.56	RSD% = 2.22	RSD% = 2.20
Vioxx tablet 12.5 mg	12.36 ± 0.025	12.16 ± 0.145		
B.N. ABR 2994	RSD% = 0.20	RSD% = 1.19	–	–
Vioxx tablet 25 mg	24.58 ± 0.713	24.72 ± 0.328		
B.N. 29802	RSD% = 2.9	RSD% = 1.29	–	–

[a] Mean of two experiments.

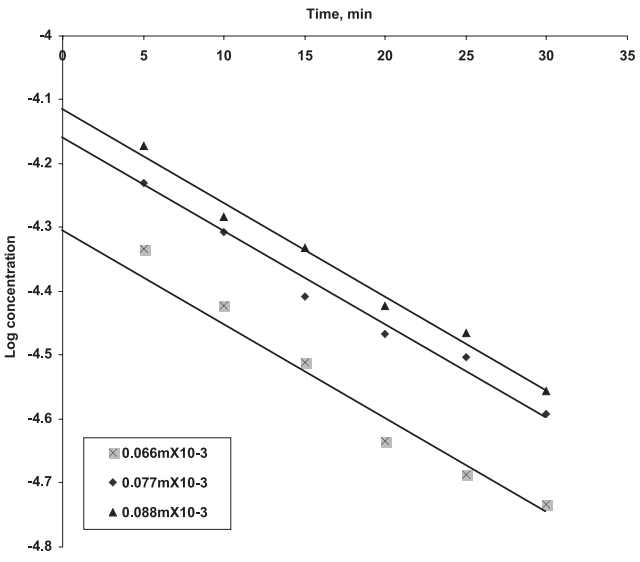


Fig. 4. Kinetic graphs of photo-degradation of rofecoxib.

of interfering substance is canceled. Accordingly, the choice of the wavelength selected for calibration is not critical. The spectra are presented in Fig. 3. Several divisor concentrations 5, 10, 15 and 20 μg ml^{-1} of degradate were tried. The best results were obtained when using 10 μg ml^{-1} of degradate as a divisor. The wavelength 284 nm was chosen as analytical λ where no noise was observed from the divisor. Both ^{1}D and ^{2}D ratio spectra (second derivative of ratio spectra) were tried ^{1}D gave more precise results. The results obtained for the analysis of rofecoxib by the ^{1}D and ^{1}DD methods are presented in Table 2. Both methods were checked by analysis of laboratory prepared mixtures of (I) and (II) in different ratios (Table 1). It was found that (I) could be determined in presence of up to 80% of (II) by the ^{1}DD. The results are given in Table 1. The methods were also applied for the analysis of rofecoxib in bulk and pharmaceutical formulations as described in Tables 3 and 4.

3.3. Method validation for ^{1}D and ^{1}DD

For the UV-spectrophotometric methods, placebo samples were prepared according to the manufacturer's formula. The spiked placebos were tested at five levels 30%, 50%, 66%, 100% and 130% of the labeled amounts of the drug. Assays were performed in duplicate on two samples of each of the five levels. No interference resulted from placebo during analytical procedures as shown in Table 3. The standard addition technique was also used to assess the accuracy of the methods either by addition of (I) or (II) as given in Table 4. Both inter- and intra-day assays were applied to check the repeatability and precision. The accuracy of the two methods is shown in Table 2. A validation sheet is also presented in Table 6.

3.4. Kinetic study

^{1}D method was used to determine the order of the photo-degradation rate of reaction by following the decrease in concentration of (I) either by measuring the peak amplitude at 316.3 nm or by determining the increase in concentration of (II) as indicated by the peak amplitude at 273.1 nm. Three different concentrations, 0.066, 0.077 and 0.088 × 10^{-3} M were used for the study. Fig. 4 is a plot of log concentration vs. time. The slope (S) of the obtained curves was nearly the

Table 5
Kinetic data of photo-degradation of rofecoxib

Concentration × 10^{-3} M	K (mol min^{-1})	t (min)	Regression equation
0.066039	0.0343	20.2	$y = -0.0147x - 4.305$, $r = 0.9811$
0.07777	0.0336	20.62	$y = -0.0146x - 4.16$, $r = 0.9909$
0.08815	0.0336	20.62	$y = -0.0147x - 4.115$, $r = 0.9939$

y, peak amplitude at the selected wavelength; x, remaining rofecoxib concentration.

same, which proves that the reaction follows first-order kinetics. The rate of degradation (K) and the t 1/2 of the reaction were calculated from the equation [12]: $S = K/2.303$ while t 1/2 $= 0.693/K$. The results are presented in Table 5.

3.5. Fluorimetric assay

The suggested method depends on the fact that (II) shows intense fluorescence [4]. Subjecting very dilute solutions of (I) in acetonitrile/water (1:1) to UV irradiation; results in the conversion of (I) to (II). The fluorescence characteristics of (II) were obtained at λ excitation and λ emission 247 and 377 nm, respectively, as shown in Fig. 5. On the other hand, (I) does not show any fluorescence. To ensure that all (I) was converted to (II), solutions in concentration range 20–1000 ng ml^{-1} were subjected to UV irradiation using UV-lamp at a distance of 15 cm for variable time from 2 to 10 min at 1-min interval. It was found that the fluorescence intensity of solutions increases up to 3 min and remained constant up to 10 min. Thus, subjecting the sample solutions to irradiation for 5 min was sufficient for complete conversion of (I) to (II) in the specified concentration ranges. The linearity range of the method was found to be from 25 to 540 ng ml^{-1} which allowed the method to be applied successfully for the determination of (I) in biological fluids as well as in bulk powder and pharmaceutical formulations (Table 7). Applying the standard addition technique on pharmaceutical formulations as presented in Table 7 assessed the validity of the method. A validation sheet is also given in Table 6.

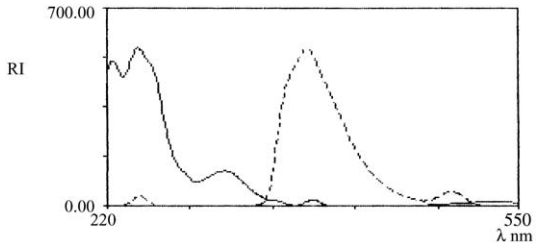


Fig. 5. Excitation (__) and emission (_ _ _) spectra of rofecoxib photo-degradate (500 ng ml^{-1}) in acetonitrile/water (1:1).

Attempts to use the method as stability indicating one by directly measuring the fluorescence of the photo-degradate (II) in presence of (I) were done. The attempts were unsuccessful since variable results were obtained upon fluorimetric measurement of very dilute solutions.

4. Conclusion

Rofecoxib could be determined by different analytical techniques. The methods have the advantage of being stabil-

Table 6
Assay validation sheet of the proposed methods for the determination of rofecoxib (I) and its photo-degradate (II)

Parameter	^{1}D at 316.4 nm for (I)	^{1}DD at 284.6 nm for (I)	^{1}D at 273.1 nm for (II)	Fluorimetric method for (I)
Accuracy (mean ± S.D.)	100.07 ± 0.84	100.06 ± 1.06	99.76 ± 1.20	101.48 ± 1.525
Precision				
Repeatability	101.33 ± 0.125	100.76 ± 1.121	99.73 ± 0.359	99.75 ± 1.102
Reproducibility	98.38 ± 0.376	100.40 ± 0.938	99.73 ± 0.376	99.75 ± 1.102
Linearity				
Slope	0.0905	0.1915	0.3975	0.993
Intercept	–0.0219	–0.1161	+0.24	+5.418
Correlation coefficient	0.9997	0.9998	1	0.9998
Range	5.88–26.2 μg ml^{-1}	5.82–26.2 μg ml^{-1}	5.12–20.48 μg ml^{-1}	25–540 ng ml^{-1}

Table 7
Results of fluorimetric assay of rofecoxib

Accuracy		Standard addition		
Taken (ng ml^{-1})	% Found (ng ml^{-1})	Preparation	Mean found (mg) ± RSD%	Recovery (%) ± RSD%
25	102.40	Vioxx tablet 12.5 mg [a]	12.45 ± 0.032	99.94 ± 1.389
50	103.20			
100	101.90	Vioxx tablet 25 mg [b]	25.15 ± 0.248	100.6 ± 0.99
200	100.52			
250	99.38	Human plasma [c]		103.9 ± 0.141
Mean ± RSD%	101.48 ± 1.505			

[a] Batch No. ABR 2994.
[b] Batch No. 29801, 29802.
[c] Equation: $y = 0.9869x - 72.665$, $r = 0.9931$.

ity indicating for the drug in presence of its photo-degradate. In addition, a kinetic study of the drug degradation was done. Being about 100 times more sensitive, the fluorimetric method is more advantageous than UV-spectrophotometric methods. It allows the analysis of the drug at very low-concentration range, which is suitable for its determination in biological fluids.

References

[1] M.C. Allison, M.G. Howatson, C.I. Torrance, F.D. Lee, R.I. Russell, NSAIDs induced gastric ulcers and hemorrhagia upon chronic use, New Engl. J. Med. 327 (1992) 749–754.

[2] F.L. Lanza, M.F. Rack, D.A. Callison, et al., A pilot endoscopic study of the gastroduodenal effects of SC-58635, a novel cox-2-selective inhibitor, Gastroenterology 112 (1997) A194.

[3] B. Mao, A. Ibrahim, Z. Ge, D. Ellison, R. Hartman, S. Prabhu, et al., Examination of rofecoxib solution decomposition under alkaline and photolytic stress conditions, J. Pharm. Biomed. Anal. 28 (2002) 1101–1113.

[4] E. Woolf, I. Fu, B. Matulszewski, Determination of rofecoxib, a cyclooxygenase-2-specific inhibitor in human plasma using HPLC with post-column photochemical derivatization and fluorescence detection, J. Chromatogr. B 730 (1999) 221–227.

[5] F. Jamali, S. Sattari, HPLC determination of cycloxygenase II inhibitor rofecoxib in rat and human plasma, J. Pharm. Pharm. Sci. 3 (2000) 307–312.

[6] C.Z. Matthews, E.J. Woolf, B.K. Matuszewski, Improved procedure for the determination of rofecoxib in human plasma involving 96-well solid-phase extraction and fluorescence detection, J. Chromatogr. A 949 (2002) 83–89.

[7] T. Radhakrishna, D. Sreeniva, G. Om Reddy, LC determination of rofecoxib in bulk and pharmaceutical formulations, J. Pharm. Biomed. Anal. 26 (2001) 617–628.

[8] K.V. Krishna, J.B. Moses, P.K. Dubey, B.S. Chandra, G. Om Reddy, K. Vyas, Isolation and characterization of process related impurities in rofecoxib, J. Pharm. Biomed. Anal. 29 (2002) 355–360.

[9] C.M. Chavez-Eng, M.L. Constanzer, B.K. Matuszewski, HPLC-tandem mass spectrometric evaluation and determination of stable isotope labeled analogs of rofecoxib in human plasma samples from oral bio-availability studies, J. Chromatogr. B 767 (2002) 117–119.

[10] U. Werner, D. Werner, R. Mondkowski, M. Gillich, K. Brune, Selective and rapid LC–MS method for the quantification of rofecoxib in pharmacokinetic studies with humans, J. Chromatogr. B 760 (2002) 83–90.

[11] Y.H. Kiang, W. Xu, M.J. Kaufman, Ab initio structure determination of rofecoxib from powder diffraction data using molecular packing analysis method and direct space method, Int. J. Pharm. 252 (2003) 213–223.

[12] A. Martin, J. Swarbrick, A. Cammarata, Physical Pharmacy, third ed, Lea and Febiger, Philadelphia, 1983, pp. 359–360.