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LC determination of rofecoxib in bulk and pharmaceutical formulations☆

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Abstract

An isocratic reversed phase-liquid chromatographic (RP-LC) method has been developed for the determination and purity evaluation of rofecoxib in bulk and pharmaceutical dosage forms using photodiode array detection set at 225 nm. The method is simple, rapid and selective. The method is capable of detecting all process intermediates and other related compounds, which may be present at trace levels in finished products. Hence the method is very useful for process monitoring during the production of rofecoxib. Chlorophenyl methyl sulphone has been used as internal standard for the quantitative determination of rofecoxib. The method is linear in the range of 125–500 μg. The precision for inter- and intra-day assay variation of rofecoxib is below 1.6% relative standard deviation (R.S.D.). The accuracy determined as relative mean error (R.M.E.) for the intra-day assay is within ± 2.0%. The drug was extracted from tablets (Vioxx) using acetonitrile. The percentage recoveries from dosage forms were ranged from 98.2 to 102.6.

Keywords: Rofecoxib; Related compounds; Purity; Assay; Chlorophenyl methyl sulphone; Formulation; RP-LC

1. Introduction

Cyclooxygenase (COX) enzyme is involved in the synthesis of prostaglandins in man. Prostaglandins are known to have a cytoprotective function in gastrointestinal mucosa as well as to play a role in the body's response to inflammation. There are two isoforms of COX in man, termed as COX-1 and COX-2 [1]. COX-1 is present in most of the tissues throughout the body and COX-2 is found in high levels at sites of inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) which are currently marketed, known to inhibit both COX-1 and COX-2 [2]. These NSAIDs relieve symptoms of inflammation and they also inhibit the synthesis of the prostaglandins that are required to protect the gastrointestinal mucosa, resulting, in the formation of ulcers in the gastrointestinal tracts of patients who are chronic users of these compounds. Thus, a drug that inhibited COX-2 without affecting COX-1 would be expected to be anti-inflammatory and lack the gastrointestinal side effects that are common to other drugs those inhibit both isoforms of COX.

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Rofecoxib [4-(4-methanesulfonylphenyl)-3-phenyl-5H-furan-2-one, Fig. 1], a new COX-2 specific inhibitor developed by Merck & Co. It was approved for the treatment of osteoarthritis and for the relief from acute pain. So far one liquid chromatography procedure has been described for the determination of rofecoxib using post-column photochemical derivatization and fluorescence detection in human plasma [3]. However, there are no other publications concerning the analysis of rofecoxib in bulk drug and in pharmaceutical dosage forms. So it is felt necessary to develop a liquid chromatographic (LC) procedure which would serve as a rapid and reliable method for the determination of rofecoxib in bulk drug and as well as in pharmaceutical formulations. In this paper, a simple isocratic reversed-phase (RP) LC method has been described for the qualitative and quantitative determination of rofecoxib. In the described method, all the possible process intermediates were well separated and eluted before 20 min run time. It is likely that the unreacted intermediates and their precursors may remain as impurities in the final active pharmaceutical ingredient (API) and affect its quality. Therefore, separation and determination of rofe-

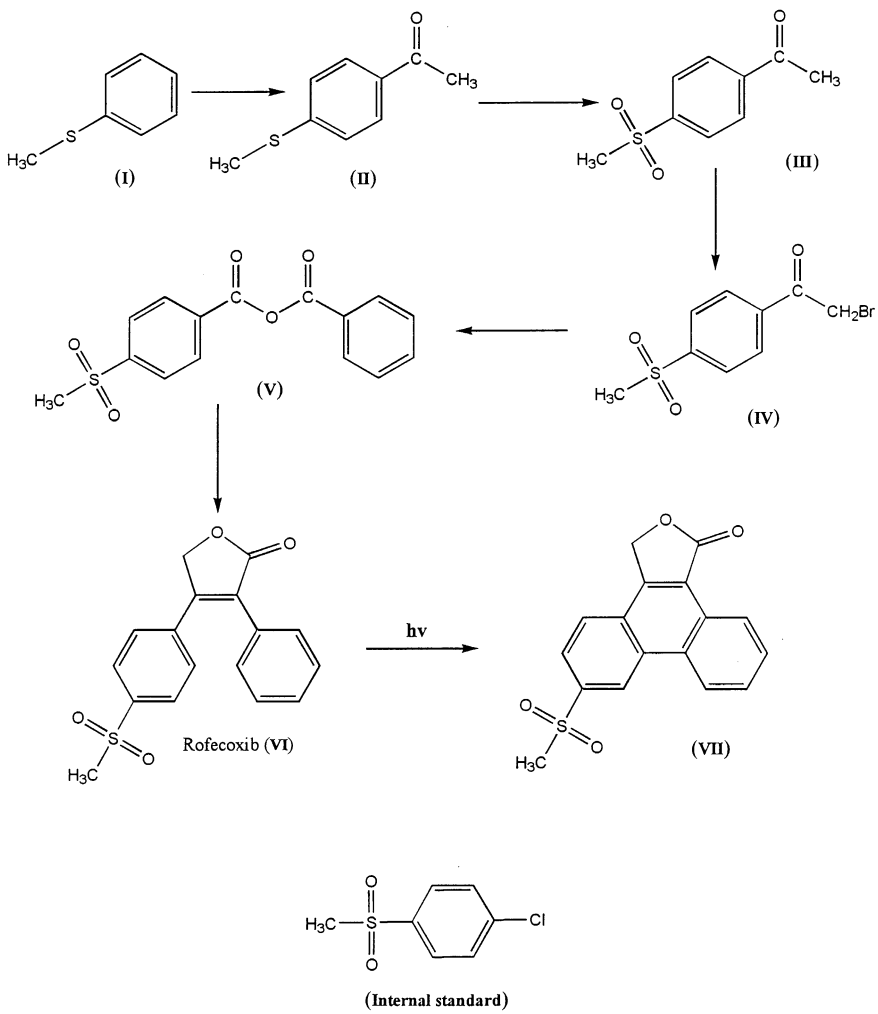

Fig. 1. Chemical structures of rofecoxib, its related compounds and internal standard.

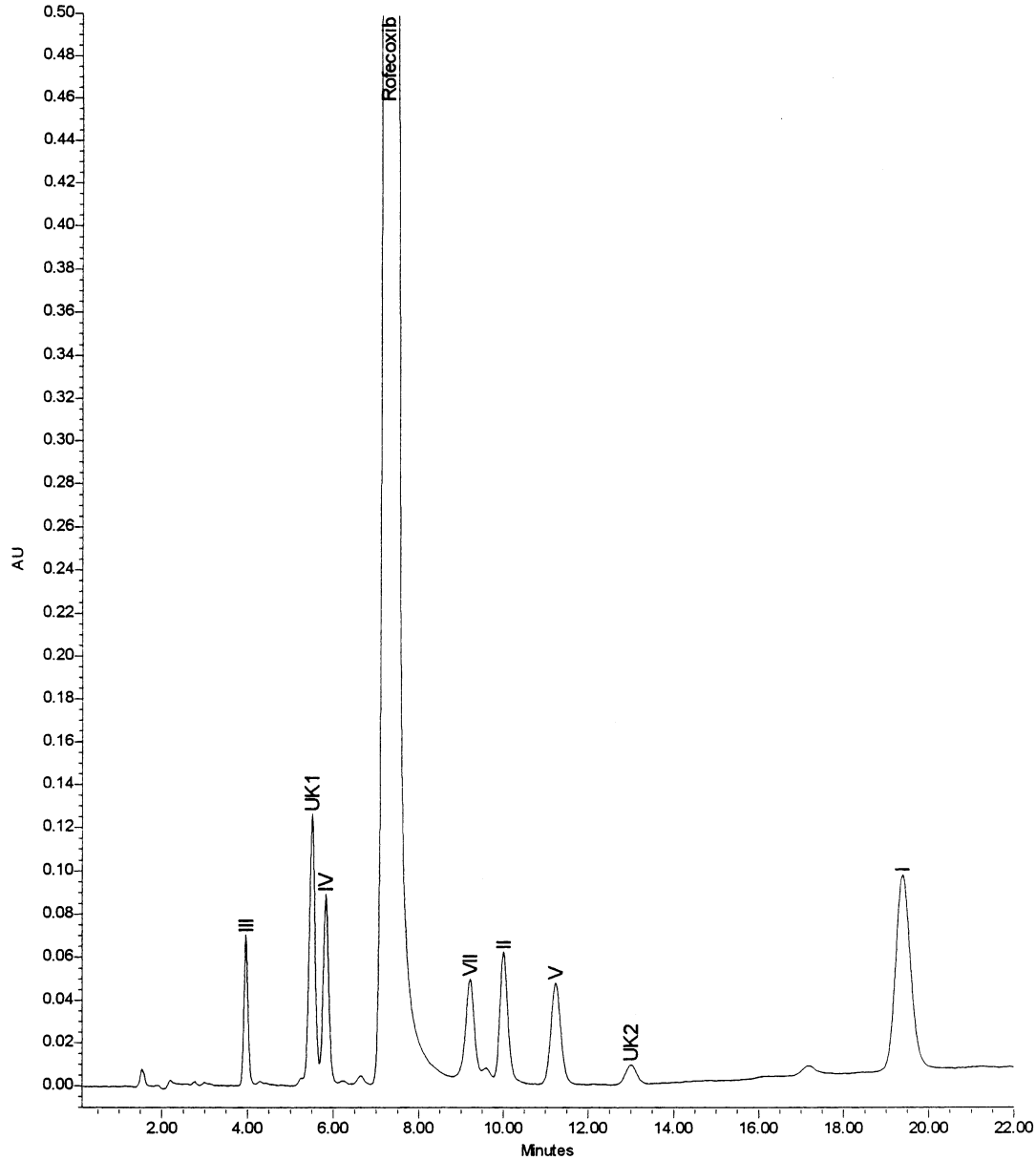

Fig. 2. HPLC chromatogram of rofecoxib and its related compounds.

coxib and its process related impurities/intermediates is important not only for quality assurance but also for process development. This particular approach has been found to be useful to control the level of impurities in the finished products. Chlorophenyl methyl sulphone (Fig. 1) was used as internal standard for the purpose of quantitation. The developed method is also found to be efficient for rofecoxib assay in pharmaceutical formulations. Finally, the method was thoroughly validated for the assay determination of rofecoxib.

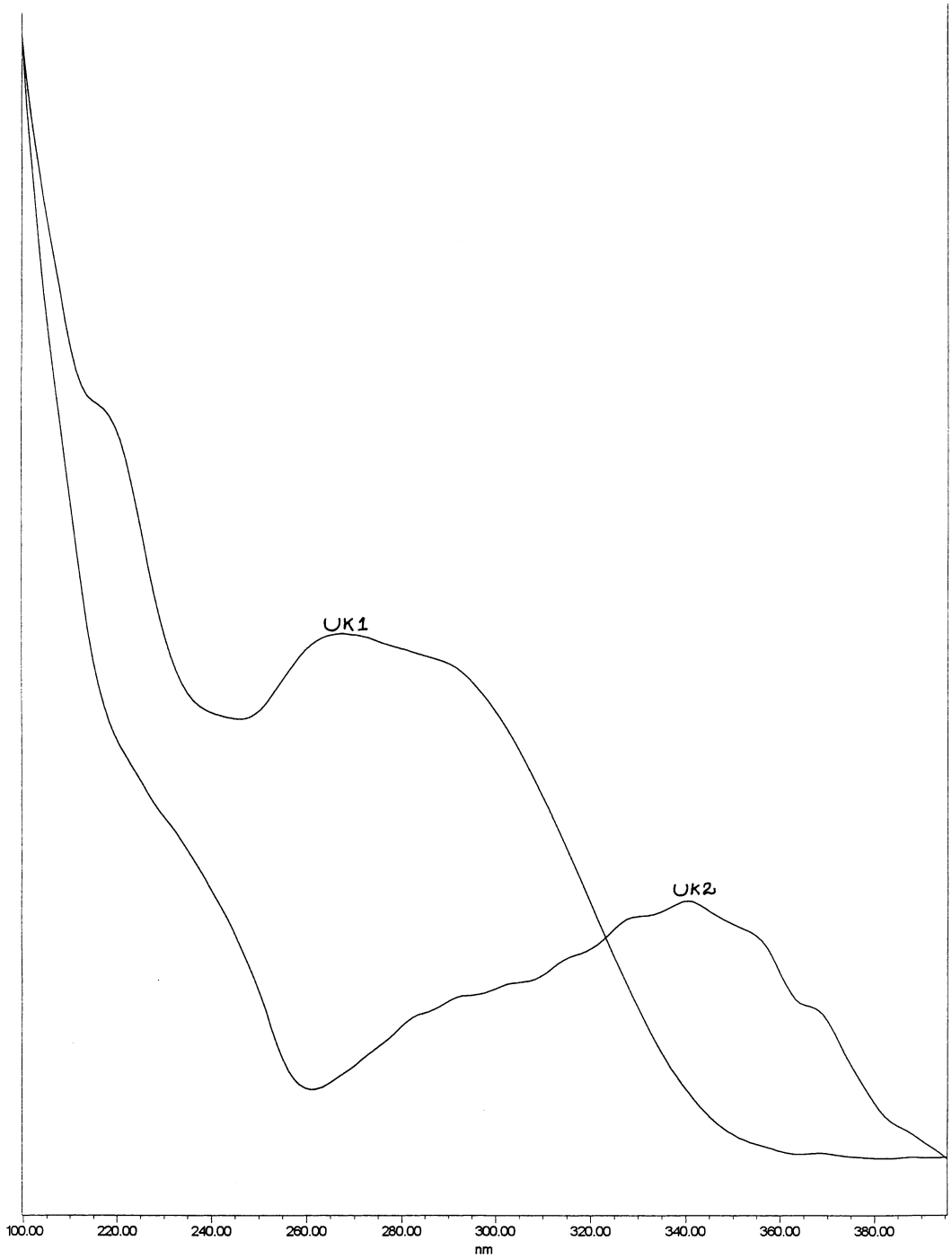

Fig. 3. Absorption spectra of unknown impurities **UK1** and **UK2** using PDA detection.

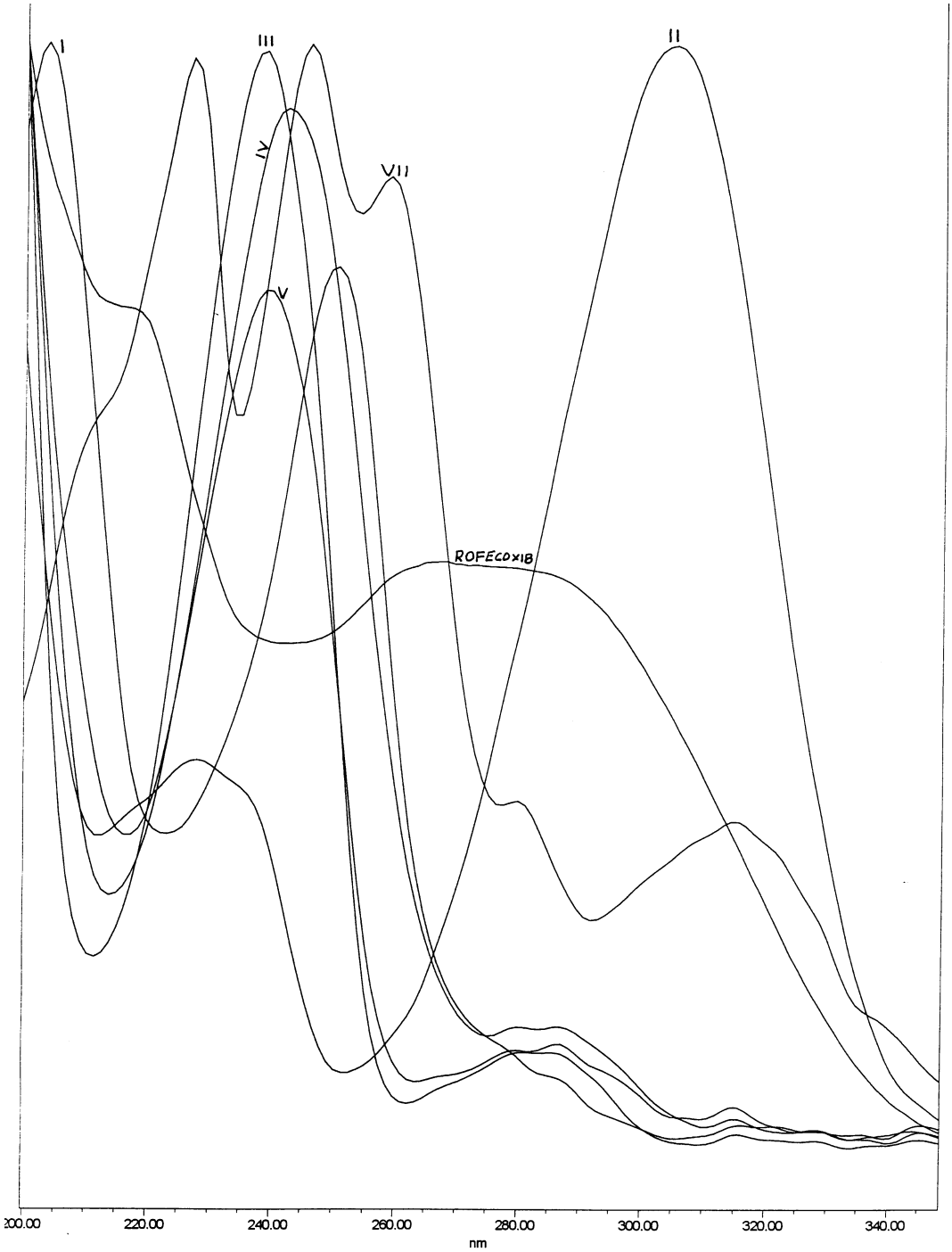

Fig. 4. Absorption spectra of rofecoxib and its process intermediates.

2. Experimental

2.1. Materials

Samples of rofecoxib and its process impurities were received from Process R&D division of Dr Reddy's Research Foundation, Hyderabad, India. High-performance liquid chromatographic (HPLC) grade acetonitrile was obtained from Merck, USA. Tablets of Vioxx were procured from Merck & Co., USA. Each tablet weighing 206.1 mg consists of 25 mg of API and the rest are excipients namely lactose, microcrystalline cellulose, hydroxypropyl cellulose, croscarmellose sodium, magnesium stearate and yellow ferric oxide. High purity water was prepared by using Waters Milli-Q plus purification system.

2.2. Instrumentation

The LC system consisted of a Waters 510 pump, a Rheodyne injector equipped with a 20 μl sample loop, and a Waters 996 Photodiode array detector. The Waters HPLC system LC Module I Plus consisted of a pump and an ultra violet–visible (UV–vis) tunable variable wavelength detector and was used in laboratory B. The output signal was monitored and integrated using Millenium 2010 Chromatography Manager software (Waters) in both lab A and lab B. Analysis was

Table 1
System suitability report

Compounds	Capacity factor[a]	Selectivity[a]	Tailing factor[a]	Resolution[a]	Theoretical plates[a]
III	0.98	–	1.087	–	8904
UK1	1.75	1.79	1.008	7.38	8219
IV	1.92	1.1	1.051	1.35	10 094
Rofecoxib	2.63	1.38	1.205	4.43	12 257
VII	3.61	1.37	1.025	5.1	10 918
II	4.00	1.11	1.155	2.24	14 166
V	4.62	1.15	1.078	3.20	11 514
UK2	5.50	1.19	1.012	3.81	11 115
I	8.69	1.58	1.149	11.16	14 770

[a] Number of samples analyzed is three.

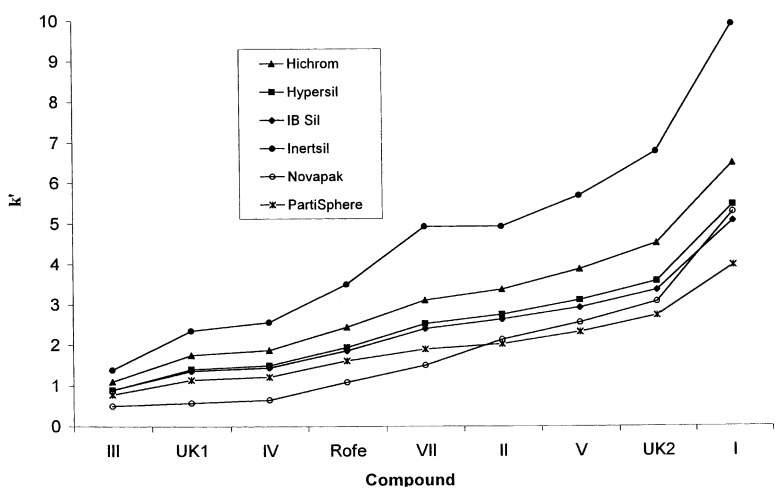

Fig. 5. Retention behavior of rofecoxib and its related compounds on equivalent columns of different manufacturers.

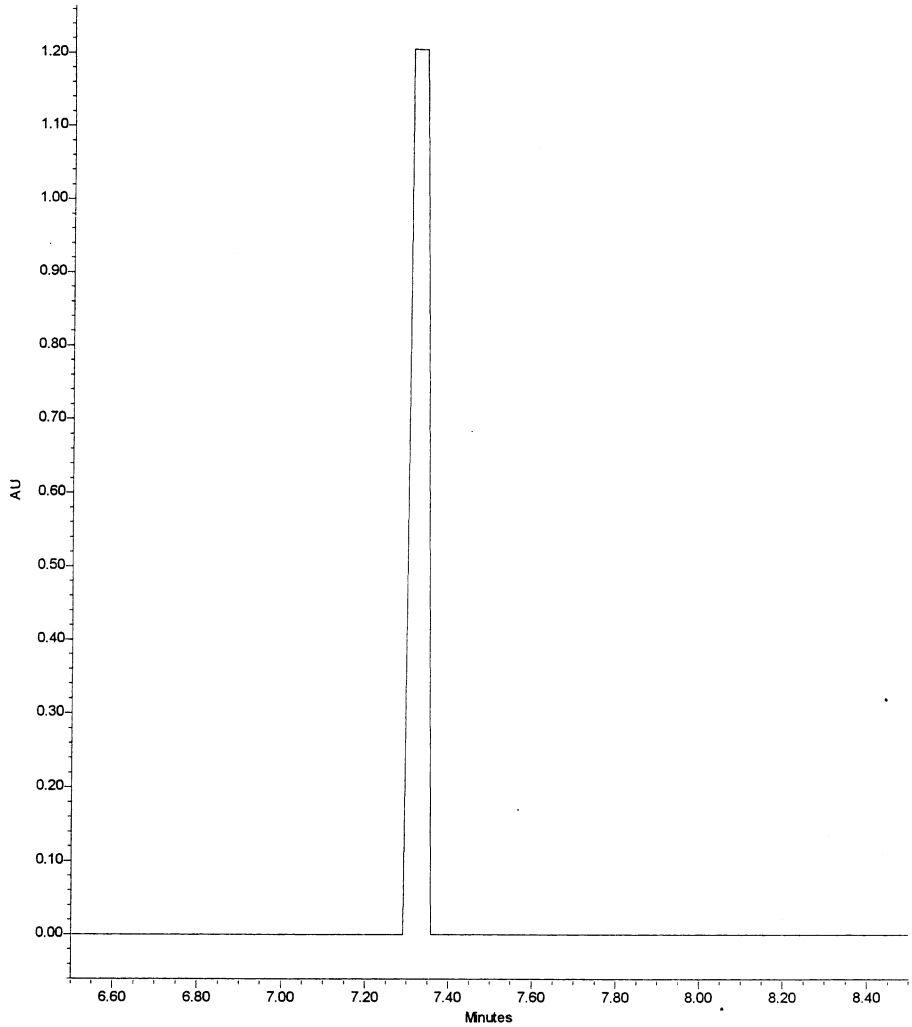

Fig. 6. Ratio-plot (225/272 nm) of rofecoxib sample using PDA detection for peak purity determination.

carried out using a Symmetry C18, 5 μm, 4.6 × 250 mm column (Waters make).

2.3. Chromatographic conditions

The mobile phase consisted of a mixture of acetonitrile–water (50:50, v/v). The mobile phase was filtered through a 0.45-μm nylon membrane filter prior to use. A Symmetry C_{18} analytical column (250 × 4.6 mm, 5 μm packing) was used for the separation. The mobile phase was delivered through the column at a flow rate of 1.0

Table 2
Assay values of rofecoxib from Vioxx tablets

Amount taken (mg; $n = 3$)	Amount recovered (mg)	Recovery (%)
0.154	0.158	102.6
0.220	0.216	98.2
0.286	0.290	101.6
0.352	0.350	99.4
0.440	0.445	101.2

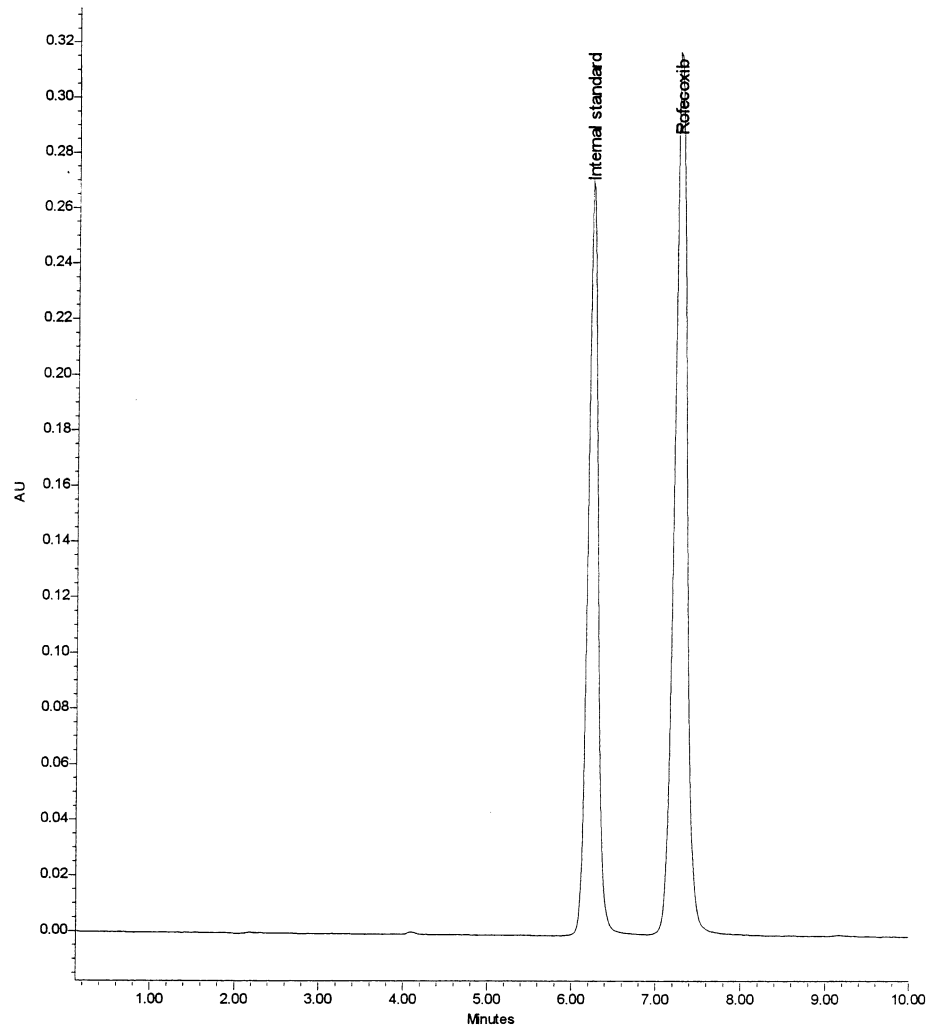

Fig. 7. HPLC chromatogram of formulated rofecoxib (Vioxx-25 mg) with internal standard.

ml/min the column was operated at ambient temperature (~ 22 °C). The sample injection volume was 10 μl. The photodiode array detector was set at a wavelength of 225 nm.

2.4. Preparation of samples

Sample solutions of rofecoxib and impurities were prepared with acetonitrile. The concentration of the internal standard was 150 μg/ml in each solution of rofecoxib and was used for validation studies. All standard flasks containing sample solutions of rofecoxib should be protected from light by wrapping them with black mask or aluminum foil.

3. Results and discussion

3.1. Separation

The structures of rofecoxib and all its process related compounds (**I**, **II**, **III**, **IV** and **V**) are given in Fig. 1. Compound **VII**, shown in Fig. 1 is a

Table 3
Accuracy in the assay determination of rofecoxib

Day of analysis	Taken (mg)	Recovery (mg; $n=3$)	% Recov.	R.M.E. (%)	T.E. (%)
0 Day	0.212	0.211	99.5	−0.47	0.03
	0.305	0.307	100.7	0.66	1.6
	0.423	0.421	99.5	−0.47	0.37
1 Day	0.212	0.212	100.0	0	0.62
	0.305	0.308	101.0	0.98	1.6
	0.423	0.422	99.8	−0.24	0.76
2 Day	0.212	0.213	100.5	0.47	0.97
	0.305	0.309	101.3	1.31	1.83
	0.423	0.425	100.5	0.47	1.37

% Recov., Percent recovery of rofecoxib from the sample against taken; R.M.E., relative mean error; T.E., total error; R.M.E. = {(MEAN−TCONC)/TCONC}100; TE = {2 S.D.+(MEAN−TCONC)/TCONC}100, in which S.D. is the standard deviation of the mean of triplicate of each concentration level and TCONC the theoretical concentration of the analyte of each level.

degraded product, which was formed upon exposure rofecoxib to UV light. This cyclized product was observed as a potential impurity in rofecoxib sample. Thus, the mobile phase was optimized to obtain the best separation and resolution between all these compounds. When the mobile phase consisting of water and acetonitrile in the ratio of 55:45, peaks of compound **VII** and **II** were merged and co-eluted. The same was observed even when the mobile phase of water:acetonitrile (60:40, v/v) was used. However, there was a good separation of these compounds when the above solvent mixture was in the ratio of 50:50. A flow rate of 1.0 ml/min with the same mobile phase allowing adequate separation of all the compounds using a Symmetry C18 column. The typical chromatogram of crude rofecoxib spiked with all related substances recorded using the described method is shown in Fig. 2. The impurities **UK1** and **UK2** have not been identified. Overlaid absorption spectra of unknown impurities **UK1** and **UK2** (absorption bands shown at 271.5 and 339.3 nm, respectively) are given in Fig. 3. In the presented method, the selectivity was found to be more than 1.1 with a resolution more than 1.35 for the case of all the compounds. System suitability results of the method are presented in Table 1. Rofecoxib and its related compounds show significant UV absorbance at wavelength 225 nm (Fig. 4). Hence this wavelength has been chosen as detection for the analysis of rofecoxib.

3.2. Column selection

Selectivity of the method was checked by using six different columns of different manufactures, which are having same stationary phase as C18. The columns used for study were, (1) Shandon Hypersil C_{18}; (2) Phenomenex IB Sil C_{18}; (3) Hichrom Hichrom RPB; (4) Whatman Parti-

Table 4
Inter- and intra-day assay variation of rofecoxib

Intra-day			
0 *Day*			
Mean of concentration (mg/ml; $n=3$)	0.211	0.307	0.421
S.D.	0.0025	0.0047	0.0042
R.S.D. (%)	1.19	1.54	0.99
1 *Day*			
Mean of concentration (mg/ml; $n=3$)	0.212	0.308	0.422
S.D.	0.0031	0.0032	0.005
R.S.D. (%)	1.44	1.04	1.18
2 *Day*			
Mean of concentration (mg/ml; $n=3$)	0.213	0.309	0.425
S.D.	0.0025	0.0032	0.0045
R.S.D. (%)	1.18	0.86	1.06
Inter-day			
Mean (of mean concentration of 3 days)	0.212	0.308	0.423
S.D.	0.001	0.001	0.0021
R.S.D. (%)	0.47	0.32	0.49

Sphere C_{18}; (5) GL. Sciences Inc. Inertsil ODS 3V; and (6) waters Novapak C_{18} with same column dimensions. A sample containing rofecoxib and all its related impurities was selected to check the selectivity. The separation between the pairs of **UK1**, **IV** and **VII**, **II** was not good when the IB Sil column used. On PartiSphere column the compounds **VII**, **II** and **V** were co-eluted. Compounds **VII** and **II** merged and co-eluted with Inertsil column. But with Novapak column the compounds **II** and **V** were eluted as single peak. The resolution between **UK1** and **IV** on both Hichrom and Hypersil columns was found to be 1.4 and 1.2, respectively, and these columns can be used as alternative for Symmetry column. The retention behavior of all the compounds on each column is shown in Fig. 5.

3.3. Assay determination

Rofecoxib can be determined quantitatively using chlorophenyl methyl sulphone as internal standard. The assay method was extensively validated using the following parameters.

3.3.1. Specificity

Complete resolution of rofecoxib from its related compounds, with no apparent shoulders (Fig. 3) confirms the specificity of the described method.

Photodiode array detection technique was used as evidence of the specificity of the method, and to evaluate the homogeneity of the peak. Chromatographic peak purity was determined using wavelength comparison (225 vs. 272 nm) [4]. The plot (Fig. 6) with flat top showed that rofecoxib exhibited a homogeneous peak with no detectable impurities embedded in it.

Accelerated degradation studies were performed to demonstrate the validity of the method. Two separate solutions of pure rofecoxib (0.25 mg/ml in mobile phase) were prepared. One solution was exposed to UV light (254 nm) for 24 h, and the other was kept at 50 °C temperature for 48 h. The degraded samples were analyzed. Rofecoxib exposed to UV light was mostly converted to cyclized compound **VII**. On the other hand, the heated rofecoxib sample does not give any major degradation products except a small peak, which corresponds to compound **VII**. Both degraded samples (UV and 50 °C) were subjected to photo-diode array analysis for peak purity. The plot report showed that rofecoxib had no detectable impurity peaks embedded in them and are free of co-eluting degradation compounds. It is clear that the method can be used for determining the stability of rofecoxib in bulk and pharmaceutical formulations.

For further demonstration of specificity, all the possible known impurities discussed above were added to pure rofecoxib sample and the mixture was analyzed for assay and the results were compared with Pure sample results. Reproducibility was observed in both the cases (relative standard deviation (R.S.D.) < 2.0).

3.3.1.1. Assay determination of rofecoxib from Vioxx tablets. The specificity of the method was also checked for a dosage form of rofecoxib. Therefore, 20 weighed tablets of Vioxx (equivalent to 25 mg of rofecoxib in each tablet) were ground to a fine powder. The amount of powder equivalent to 50 mg of the active compound was extracted with acetonitrile and centrifuged. The supernatant was diluted with acetonitrile to required concentrations and analyzed. The results of the extracted samples at five different independent concentrations were comparable with claimed values (Table 2). Rofecoxib and the internal standard were well separated from the excipient peaks in the formulation sample. A typical LC chromatogram is shown in Fig. 7.

3.3.2. Linearity

Linearity was checked by preparing standard solutions at seven different concentration levels ranging from 125 to 500 μg/ml. The linearity was also checked for 3 consecutive days for the solutions of same concentrations prepared from the stock solution. The precision for inter-day linearity is below 1.2% R.S.D. The equation for calibration curve is $y = 4.43x + 0.034$ and the

standard error (S.E.) is 0.0157. The correlation coefficient was found to be more than 0.999, indicating good linearity.

3.3.3. Accuracy

The accuracy of the method was checked at three concentration levels i.e. at 200, 300, and 400 μg each in triplicate. The assay accuracy was tested for 3 days at the same concentration levels in triplicate. Solutions for the standard curves were prepared fresh every each day. The assay accuracy variation shown in terms of relative mean error (R.M.E.), total error (T.E.) and percent recovery are tabulated in Table 3 [5]. The R.M.E. and T.E. values are below ± 2.0 for the intra-day assay experiments.

3.3.4. Precision

The assay precision was studied for the parameters viz. repeatability, intermediate precision and reproducibility (ruggedness). Repeatability is the intra-day variations in assay values obtained at different concentration levels of rofecoxib are expressed in terms of R.S.D. values calculated from the data of each day of 3 days i.e. 0–2 days.

R.S.D. values were found to be well below 2.0%, indicating a good repeatability (Table 4).

The intermediate precision is the inter-day variation at the same concentration level, is determined on successive days. The inter-day variations calculated for each concentration level from 3 days data are expressed in terms of R.S.D. values. At each concentration level, the R.S.D. values were well below 1% indicating a good intermediate precision (Table 4).

The ruggedness of an assay method is defined as the degree of reproducibility of the results obtained by analysis of the same sample under a 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 2 were analyzed at laboratory B with a different instrument (LC Module I plus HPLC system containing Pump and a UV–vis detector) by a different analyst with different lots of reagents and another batch column. The data obtained from laboratory B were within 2% R.S.D. when compared with the data of parent lab.

3.3.5. Stability

In routine testing in which many samples are prepared and analyzed every day, it is essential that solutions are stable enough to allow for delays such as instrument breakdowns or overnight analysis using auto samplers. The stability of rofecoxib in solution containing acetonitrile and the internal standard was determined for the samples stored at normal light conditions (tightly capped flasks kept on bench top) and under reduced light conditions (tightly capped flasks were wrapped with aluminum foil). The samples were checked for 3 days of storage and the data was compared with freshly prepared samples. Solutions kept under reduced light conditions were found stable and the R.S.D. values of assay were well below 2.0% against freshly prepared samples. The rofecoxib solutions kept under normal light conditions are getting degraded to give cyclized impurity with time.

4. Conclusion

An analytical RP-LC method developed and validated for the purity and quantitative determination of rofecoxib in bulk and as well as in pharmaceutical dosage forms has been described. The developed method has been found to be selective, sensitive, precise and stability indicating. The method is also capable of detecting intermediates and other related compounds, which may be present in trace levels in finished products. Also, kinetic measurements can be carried out by this method to determine completion times for synthetic reactions.

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