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Quantitative determination of rofecoxib in pharmaceutical preparations

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Rofecoxib was assayed by UV spectrophotometry and HPLC, the concentration ranges were 2–30 $\mu g \cdot ml^{-1}$ and 5–50 $\mu g \cdot ml^{-1}$, respectively. The results, obtained by the two methods in pharmaceutical preparations were compared of each other. There were no significant differences between the mean values and the precisions.

Rofecoxib, 4-(4-methanesulfonylphenyl)-3-phenyl-5*H*-furan-2-one is a new nonsteroidal antiinflammatory drug (NSAID) with a highly selective cyclooxygenase-2 (COX-2) inhibitory action. It is used for the treatment of osteoarthritis and for the relief from pain. Rofecoxib has been determined in human plasma (Woolf et al. 1999; Chavez-Eng et al. 2000; Matthews et al. 2001; Werner et al. 2001; Chavez-Eng et al. 2002; Matthews et al. 2002) and commercial preparations (Abdel-Hamid 2000; Radhakrishna et al. 2001) by HPLC. Since the use of rofecoxib is increasing rapidly, it is essential to develop simple and suitable methods for its estimation in bulk and formulations. This report presents two methods for the quantitative determination. One of these methods is based on UV spectrophotometric detection which is simpler and more economic than HPLC. The procedure was applied successfully to the analysis of commercial tablets (Vioxx®, 25 mg) and the results were also compared with those of another HPLC method which was given by Radhakrishna et al. 2001. Some modifications were applied to this method

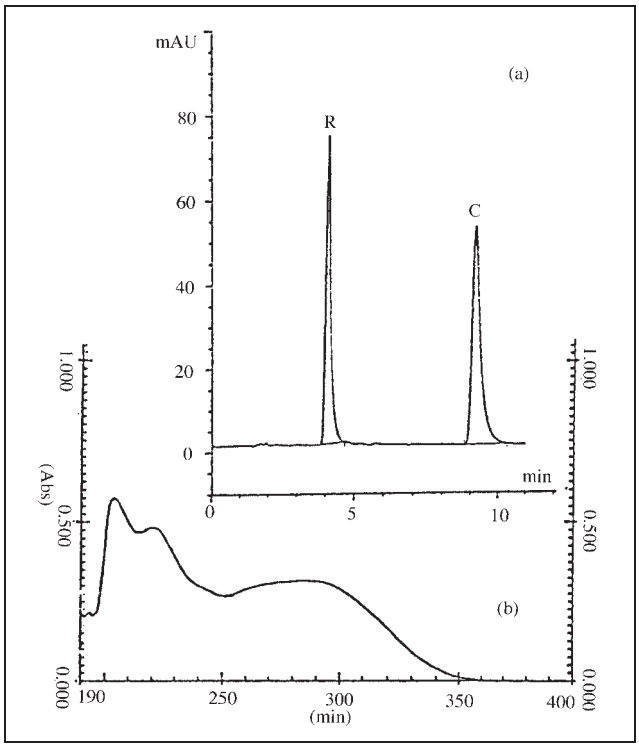

Fig: (a) Chromatogram of a tablet solution, R: rofecoxib and C: celecoxib, (b) UV spectra of rofecoxib in ethanol, c = 8 $\mu g \cdot ml^{-1}$

namely, internal standard, detector, mobil phase composition and concentration range. Under these conditions, the retention times of rofecoxib and celecoxib (internal standard) were 4.0 and 9.2 min respectively (Fig.). Concentration ranges were 2–30 $\mu g \cdot ml^{-1}$ for UV and 5–50 $\mu g \cdot ml^{-1}$ for HPLC. All standard and sample solutions were daily prepared, protected from light by wrapping them with black mask and analyzed within 1 h after preparation. The variation of acetonitrile ratio in standard solutions did not affect the linearity range of the UV method, although 5% increase was observed in the absorbency at maximum concentration of the calibration curve. However, no change was observed at minimum concentration.

The results obtained by the methods proposed were statistically analyzed and compared with those of each other. The linear regression equations were calculated for $UV_{220.2}$, $UV_{285.3}$, and HPLC methods and the correlation coefficients were found to be 0.9991, 0.9999, 0.9999, re-

Table: Experimental and statistical data

Method[a]	n	Regression equation	$se_{(b)}$[b]	$se_{(a)}$[c]	95% Conf. int. of slope	95% Conf. int. of intercept
$UV_{220,2}$	12	$A = 5.5565 \times 10^{-2}\, c + 2.0958 \times 10^{-2}$	7.8162×10^{-4}	1.1725×10^{-2}	$5.5565 \times 10^{-2} \pm 1.7414 \times 10^{-3}$	$2.0958 \times 10^{-2} \pm 2.6122 \times 10^{-2}$
$UV_{285,3}$	12	$A = 3.8696 \times 10^{-2}\, c + 5.1739 \times 10^{-4}$	1.9723×10^{-4}	3.4142×10^{-3}	$3.8696 \times 10^{-2} \pm 4.3943 \times 10^{-4}$	$5.1739 \times 10^{-4} \pm 7.6069 \times 10^{-3}$
HPLC	10	$A' = 2.6581 \times 10^{-2}\, c + 1.0733 \times 10^{-3}$	1.7494×10^{-4}	5.4275×10^{-3}	$2.6581 \times 10^{-2} \pm 4.0342 \times 10^{-4}$	$1.0733 \times 10^{-3} \pm 1.2516 \times 10^{-2}$

Method[d]	Conf. int. (t 2,571)	Recovery (%)	RSD	SEM	Comparison of the results[d]				
					Method	$t_{calc.}$	t_{table}	$F_{calc.}$	F_{table}
$UV_{220,2}$	$25.453 \pm 0.259^*$	101.81	0.9681	0.1006	$UV_{220,2}$/HPLC	0.55	2.23^*	1.23	5.05^*
$UV_{285,3}$	$25.280 \pm 0.080^*$	101.12	0.3031	0.0313					
HPLC	$25.378 \pm 0.233^*$	101.51	0.8753	0.0907	$UV_{285,3}$/HPLC	1.02	2.23^*	8.40	10.97^{**}

[a] For standards, [b] Standard error of the slope, [c] Standard error of the intercept on ordinate, [d] For tablets (n = 6), * P 0.05, ** P 0.01

spectively. The precision of the fit was confirmed from the standard error values of the slope and intercept on ordinate (Altman 1999). For the spectrophotometric and chromatographic methods, the confidence interval for the mean values, standard deviations, relative standard deviations (RSD), recoveries (%) and the standard error of the means (SEM) were calculated. The statistic results were shown in the Table. They indicated that calculated values of t are less than those of tabulated and there were no significant differences between the mean values at the 95% confidence level. The calculated values of F were found to be less than the tabulated values at the 5% significance level for UV at 220 nm and HPLC and at the 1% significance level for UV at 285 nm and HPLC. There were no significant differences between the precisions.

The results show that these methods have a good accuracy and precision. Both are simple, rapid and suitable for the estimation of rofecoxib in pharmaceutical formulations. Probably, the HPLC method presented can also be applied to determine celecoxib in pharmaceutical formulations with rofecoxib as an internal standard.

Experimental

Spectrophotometric analysis was performed on a Shimadzu UV-2100S double beam spectrophotometer using 1-cm quartz cells. Rofecoxib was kindly supplied from Yilbak Ticaret AŞ-Selectchemie AG (İstanbul, Turkey). Acetonitrile was purchased from Riedel-de Haën. Ethanol 95% was of analytical grade.

Stock solution concentration in acetonitrile was 200 $\mu g \cdot ml^{-1}$. Standard solutions which contain 2–30 $\mu g \cdot ml^{-1}$ rofecoxib were freshly prepared by diluting the stock solution with ethanol. Absorbance values of standard solutions were recorded at 220.2 and 285.3 nm.

Twenty-eight tablets were weighed and powdered. The powder prepared from tablets (204 mg) was accurately weighed, transferred into a 100 ml volumetric flask and diluted to volume with acetonitrile. The mixture was sonicated for 10 min and clarified by centrifugation. The supernatant (2–4.4 ml) was transferred into a 50 ml volumetric flask and diluted to volume with ethanol. Absorbance values were recorded at 220.2 and 285.3 nm against blank. The concentrations of rofecoxib (c) were calculated by placing the absorbance values (A) into the regression equation which was obtained from standard solutions (Table).

For the chromatographic method, a high-pressure pump Model 510 Waters was connected to a 200 µl loop injector, a Kromasil 100–5C_{18} reversed phase column, UV detector Model 481 Waters, detection at 225 nm and a chromatography data handling system Model 4880 Unicam. The peak area ratio was used for quantitation. The mobile phase was acetonitrile : water (65 : 35). Flow rate, injection volume and gain were 1,0 $ml \cdot min^{-1}$, 10 µl and 1 V, respectively. Double distilled water was freshly used. Celecoxib was kindly supplied from Yılbak Ticaret AŞ-Selectchemie AG (İstanbul, Turkey) and showed an isolated peak in the chromatogram (Fig.).

Stock solution concentrations of rofecoxib and celecoxib in acetonitril were 500 $\mu g \cdot ml^{-1}$. Standard solutions containing 5–50 $\mu g \cdot ml^{-1}$ of rofecoxib and 40 $\mu g \cdot ml^{-1}$ of celecoxib were freshly prepared diluting the stock solution with acetonitrile.

The tablet stock solution was performed as described above. After centrifugation, 3–8 ml of supernatant was transferred into a 50 ml volumetric flask. Internal standard was added and diluted to the desired volume with acetonitrile. These solutions were chromatographed and the area of each peak was determined. The concentrations of rofecoxib (c) were calculated by placing the peak area ratio (A′) into the regression equation which was obtained from standard solutions (Table).

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