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Comparison of derivative spectrophotometric and liquid chromatographic methods for the determination of rofecoxib

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Received September 10, 2003, accepted October 15, 2003

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Pharmazie 59: 453–456 (2004)

Two different UV spectrophotometric methods were developed for the determination of rofecoxib in bulk form and in pharmaceutical formulations. The first method, an UV spectrophotometric procedure, was based on the linear relationship between the rofecoxib concentration and the λ_{max} amplitude at 279 nm. The second one, the first derivative spectrophotometry, was based on the linear relationship between the rofecoxib concentration and the first derivative amplitude at 228, 256 and 308 nm. Calibration curves were linear in the concentration range using peak to zero 1.5–35.0 $\mu g \cdot ml^{-1}$. HPLC was carried out at 225 nm with a partisil 5 ODS (3) column and a mobile phase constituted of acetonitrile and water (50 : 50 v/v). A linear range was found to be 0.05–35.0 $\mu g \cdot ml^{-1}$. The developed methods were successfully applied for the assay of pharmaceutical dosage form. The statistics of the analytical data is also presented. The results obtained by first derivative spectrophotometry were compared with HPLC and no significant difference was found.

1. Introduction

Several selective COX-2 inhibitors such as celecoxib, rofecoxib and valdecoxib have been marketed as a new generation of NSAIDs. Rofecoxib (MK-0966), [4-(4-methanesulfonylphenyl)-3-phenyl-5 H-furan-2-one], is indicated for the treatment of osteoarthritis and relief of pain. A few HPLC and liquid chromatography-mass spectrometry methods have been reported for the estimation of rofecoxib in bulk form and pharmaceutical products (Aravind et al. 2002; Chavez-Eng et al. 2000; Chavez-Eng et al. 2002; Jamali and Sattari 2000; Matthews et al. 2002; Radhakrishna et al. 2001; Vallano et al. 2002; Werner et al. 2001; Woolf et al. 1999). The main problems encountered using such methods are either the need for derivatisation or the need for time-consuming extraction procedures. Since these techniques have an expensive instrumentation and running costs, the use of simpler, faster and less expensive, but still sensitive, spectrophotometric methods can be an interesting alternative, mainly those based on derivative spectrophotometry.

Derivative spectrophotometry is an analytical technique for the enhancement of sensitivity and specificity in qualitative and quantitative analysis of various compounds including pharmaceuticals and human serum (Altuntas et al. 1998, 2000; Biryol and Erk 2003; Erk 2002; Fasanmade and Fell 1985; Fell et al. 1981). Derivative spectrophotometry presents an additional advantage over spectrophotometry and chromatography in the determination of substances in pharmaceutical preparations and human plasma, since those formulations usually give turbid solutions. There is no need for time cosuming and tedious extraction processes to eliminate the excipients. In the present work, we attempted to develop an easier, accurate, and reproducible analytical method with better detection range for the determination of rofecoxib in bulk form and in pharmaceutical products.

2. Investigations, results and discussion

2.1. Spectrophotometric methods

To develop a sensitive first derivative spectrophotometric method various solvent systems were tried, such as water, methanol, 0.1 N HCl, 0.1 N NaOH and acetonitrile alone or in combinations. The final decision of using methanol was based on sensitivity, interference, ease of preparation, and cost.

The direct UV spectrum and the first derivative spectrum of rofecoxib in methanolic solutions are shown in Fig. a and Fig. b, respectively. Both spectra could be used for the determination of this drug. The UV absorption spectrum of rofecoxib in methanol showed a maximum absorbance at 279 nm. The first derivative spectrum has three peaks at $^1D_{228}$[3], $^1D_{256}$ and $^1D_{308}$ which could be used for the determination of rofecoxib. The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed, the wavelength increment ($\Delta\lambda$) over which derivative is obtained. All the parameters need to be optimized to give a well resolved large peak. Generally, the noise level decreases as $\Delta\lambda$ increases, which leads to less pronounced fluctuations in the derivative spectrum. Since spectral resolution is very poor at excessively high $\Delta\lambda$ values, the optimum value of $\Delta\lambda$ should be determined

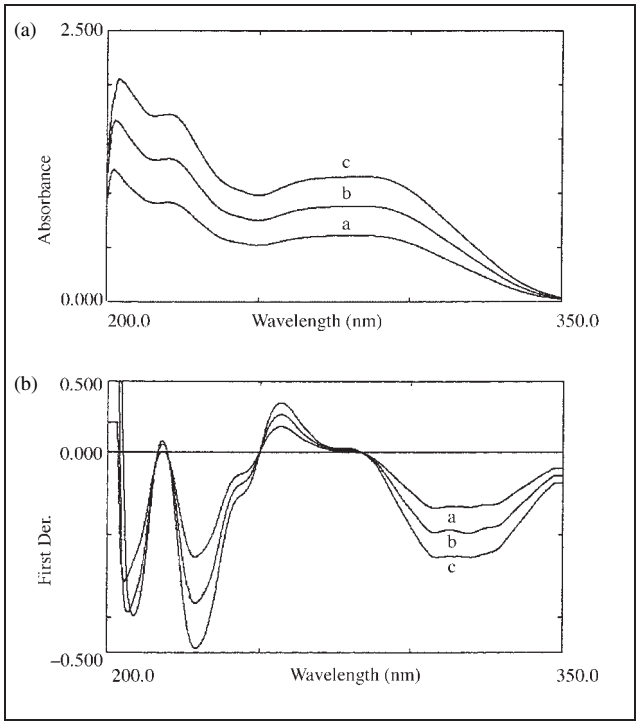

Fig.: Zero-order spectra of (a) and first derivative spectra (b) of a) 5.0 μg · ml^{-1}; b) 25.0 μg · ml^{-1} and c) 30.0 μg · ml^{-1} rofecoxib in methanol

by taking into account the noise level, the resolution of the spectrum and sample concentration. Several values of $\Delta\lambda$ were tested and 6 nm was selected as the optimum for a satisfactory signal-to-noise ratio. By measuring the values of the first derivative, the concentration of rofecoxib can be directly calculated since the first derivative measurement cancels the irrelevant absorbance due to excipients in the pharmaceutical products. The Beer's law range, linear regression equations, and correlation coefficients determined for each method are given in Table 1.

2.2. HPLC

HPLC was developed as a reference method for the developed spectrophotometric methods.

Optimum conditions were fixed by varying one parameter at a time by fixing other parameters constant and observing its effect on the response factor and also on the peak resolution. Effect of wavelength on the response factor and on the peak resolution was observed over the wavelength range 210–230 nm. 225 nm was found to be optimal. Similarly, the effect of composition of the mobile phase was studied by changing the composition of acetonitrile and water. The optimum ratio of acetonitrile: water was found to be 50 : 50 v/v. Effect of flow rate was observed by varying the flow rate from 0.8 to 1.5 ml · min^{-1}. The lower flow rates lead to increase in resolution time and high flow rates lead to considerable increase in the pressure. Therefore, 1.0 ml · min^{-1} was found to be optimal for all measurements. A RP-Phenomenex, Partisil 5 ODS (3) (250 × 4.6 mm i.d, 5 μm particle size) column is recommended because of its demonstrated ruggedness and reproducibility in this assay. Atorvastatin was applied as an internal standard. For the estimation of rofecoxib and atorvastatin sharp and symmetrical peaks were obtained with good baseline, thus facilitating the accurate measurement of the peak area. The average retention times for rofecoxib and atorvastatin were found to be 3.3 ± 0.04, and 2.1 ± 0.3 min, respectively. Under the described HPLC conditions, the respective compounds were clearly separated and their corresponding peaks were sharply developed at reasonable retention times.

2.3. Validation of the methods

The procedures were validated via evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability and recovery. The LOD and LOQ values were calculated from the calibration curves as kSD/b where k = 3 for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve. The lowest LOD and LOQ were calculated and are presented in Table 1.

Recovery studies were conducted to determine the accuracy of the proposed methods. They were performed on a synthetic mixture prepared by adding accurately weighed amounts of rofecoxib to the excipient mixture. The selectivity of the proposed methods for the estimation of the drug in presence of various tablet excipients such as starch, lactose, talc and magnesium strearate was investigated. A placebo comprising starch 10%, lactose 40%, talc 2% and magnesium strearate 1% was prepared. A 1 : 1 blend of drug and placebo was prepared.

Comparision of the original and the first derivative spectra and the chromatogram of rofecoxib in standard and drug formulation solutions showed that the wavelength of maximum absorbance and first derivative amplitudes and retention time did not change. It could be concluded that excipients did not interfere with the quantitation of rofecoxib. The above method was precise, accurate and selective (Table 2).

Table 1: Statistical analysis of calibration parameters for the determination of rofecoxib by different methods

Parameters	UV Spectrophotometry	First Derivative Spectrophotometry			HPLC
Wavelength (nm)	279.1	228.6	256.5	308.8	225.0
Limit of detection (μg · ml^{-1})	0.50	0.36	0.32	0.47	0.09
Limit of quantitation (μg · ml^{-1})	0.92	0.83	0.79	0.94	0.64
Range (μg · ml^{-1})	2.5–30.0	1.5–35.0	1.5–35.0	1.5–35.0	0.05–35.0
Regression equation (Y)[a]					
Slope (b)	2.93×10^{-2}	2.88×10^{-3}	2.47×10^{-3}	9.32×10^{-3}	3.38×10^{-1}
Std. dev. on slope(S_b)	2.27×10^{-5}	3.72×10^{-5}	2.37×10^{-4}	1.42×10^{-6}	4.03×10^{-3}
Intercept (a)	2.32×10^{-3}	3.08×10^{-4}	3.17×10^{-4}	3.15×10^{-4}	7.67×10^{-2}
Std. dev. on intercept (S_a)	1.19×10^{-6}	4.96×10^{-6}	2.36×10^{-4}	7.41×10^{-6}	1.32×10^{-4}
Std. error of estimation (S_e)	3.50×10^{-6}	2.05×10^{-5}	7.46×10^{-3}	2.27×10^{-7}	5.27×10^{-4}
Correlation coefficient (r)	0.9985	0.9999	0.9999	0.9986	0.9996

[a] Y = a + bC where C is concentration in μg · ml^{-1} (Five replicate samples)

Table 2: Assay results for the determination of rofecoxib in laboratory synthetic mixture by spectrophotometric methods and HPLC (n = 5)

Sample no	Label claim ($\mu g \cdot ml^{-1}$)	UV Spectrophotometry			
		Mean[a]	RSD (%)	Standard error	Recovery (%)
1	5.0	4.8	0.89	0.4089	96.0
2	25.0	25.1	0.78	0.9659	100.4
3	30.0	30.5	0.65	0.0094	101.7
First Derivative Spectrophotometry					
$^1D_{228}$[b]					
1	5.0	5.1	1.21	0.0892	102.0
2	25.0	24.6	1.35	0.0536	98.4
3	35.0	35.1	1.43	0.0428	100.3
$^1D_{256}$					
1	5.0	4.8	0.62	1.1312	96.0
2	25.0	24.6	0.48	1.0428	98.4
3	35.0	34.5	1.32	1.2536	98.6
$^1D_{308}$					
1	5.0	5.1	0.87	0.7258	102.0
2	25.0	24.9	0.61	0.6549	99.6
3	35.0	34.8	0.94	0.8621	99.4
HPLC					
1	5.0	4.9	0.58	0.1321	98.0
2	25.0	25.1	0.46	0.4285	100.4
3	35.0	34.9	0.35	0.2356	99.7

[a] mean in $\mu g \cdot ml^{-1}$ and RSD for five triplicate determination
[b] Order derivative$D_{\text{wavelength measured}}$

The methods were applied to the determination of rofecoxib in VIOXX® tablets. The results of the proposed methods for the same preparations were compared with HPLC chosen as reference method by means of student's t-test at 95% confidence level and no significant difference between the methods was found (Table 3).

The most striking feature of the derivative spectrophotometry is its simplicity and rapidity, without time-consuming sample preparation steps such as filtration, or degassing which are needed for HPLC procedure. The assay results obtained by these two methods are in fair agreement. In general, all the reported methods can be used for routine quality control analysis of the investigated drug in pharmaceutical preparations.

3. Experimental

3.1. Materials and reagents

Pharmaceutical grade of rofecoxib (certified to contain 99.7%) was kindly supplied by Merck Sharp & Dohme Pharm. Co. (Istanbul, Turkey) and the internal standard (IS), atorvastatin, was obtained as a gift from Sanovel Pharmaceutical Co. (Istanbul, Turkey). Vioxx® tablets (each tablet contains 25 mg of rofecoxib) were supplied from local pharmacies. All other chemicals were of HPLC grade and analytical-reagent grade.

3.2. Apparatus

A Shimadzu UV-1601 recording double-beam UV-Visible spectrophotometer with data processing capacity was used. UV spectra of reference and test solutions were recorded in 1 cm quartz cells at a scan speed of $50\ nm \cdot min^{-1}$.

The HPLC system consisted of a membrane degasser, a binary solvent delivery system, a Rheodyne injector equipped with a 20 µl sample loop, and a UV/VIS detector (1100 Series, Agilent Technologies, USA). The detection wavelength was at 225 nm, and the peak areas were integrated automatically with Windows NT based LC ChemStation Software.

The chromatographic analysis was performed at ambient temperature on a Phenomenex, Partisil 5 ODS (3) column (250 × 4.6 mm i.d, 5 µm particle size) with a mobile phase composed of acetonitrile: water (50 : 50 v/v). The flow rate was maintained at $1.0\ ml \cdot min^{-1}$.

3.3. Standard solutions and calibrations

Stock standard solutions were prepared separately by dissolving 100 mg of each drug in methanol for spectrophotometric methods and in acetonitrile for HPLC.

The standard solutions were prepared by dilution of the stock standard solutions with the same solvent to reach a concentration range of rofecoxib at 2.5–30.0 $\mu g \cdot ml^{-1}$ for UV spectrophotometry, 1.5–35.0 $\mu g \cdot ml^{-1}$ for the first derivative spectrophotometry and 0.05–35.0 $\mu g \cdot ml^{-1}$ for HPLC.

3.4. Assay procedures for tablet

An accurately weighed amount of powdered tablets equivalent to about one tablet was transferred into a 100 ml conical flask in methanol. After 30 min of mechanical shaking, the solution was filtered in a 100 ml calibrated flask through Whatman No 42 filter paper. Then the volume was completed to 100 ml with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrates and diluting them with methanol. The HPLC determination of rofecoxib was made by adding an aliquot of the above mentioned solution to the mobile phase and then these solutions were filtered through 0.45 -µm membrane filters. Triplicate 20 µl injections were made for each solution.

3 Order derivative$D_{\text{wavelength measured}}$

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Table 3: Results of the assay of rofecoxib and its commercial formulations by the proposed methods

Sample	Recovery (mean ± SD)%[a]				
	UV Spc.	$^1D_{228}$[b]	$^1D_{256}$	$^1D_{308}$	HPLC
Pure drug solution	98.3 ± 0.63 t = 1.14 (2.26)[c]	97.8 ± 1.11 1.58	99.8 ± 0.97 1.61	99.3 ± 0.62 1.52	99.9 ± 0.56
Commercial tablets[d]	99.7 ± 1.43 t = 0.97	99.8 ± 0.58 1.22	99.7 ± 0.54 0.87	99.7 ± 0.25 1.44	101.0 ± 0.42

[a] Mean and relative standard deviation for ten determinations; percentage recovery from the label claim amount
[b] Order derivative$D_{\text{wavelength measured}}$
[c] Values in parentheses are the theoretical values at p = 0.95
[d] VIOXX® tablets were labeled to contain 25.0 mg/tablet rofecoxib

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