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Determination of Etoricoxib in Bulk Drug, Dosage Form and Human Plasma by UV- Spectrophotometry

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A simple, rapid, precise spectrophotometric method for estimation of etoricoxib in bulk drug, dosage form and human plasma was developed. Sample preparation for the developed method employs 90% methanolic sodium hydroxide (0.1 M) as the solvent system for analyzing bulk drug and dosage form, while precipitation using acetonitrile (direct procedure) and liquid-liquid extraction with ethyl acetate (indirect procedure) was utilized for determination in human plasma samples. All samples were analyzed spectrophotometrically at 280 nm. For analysis of dosage form the method was found to be linear in the range of 3–60 μg/mL (r^2 = 0.9997 and 0.9998); for estimation of human plasma samples the method was found to be linear in the range of 0.1–20 μg/mL (r^2 = 0.9998 and 0.9994 respectively for direct and indirect method). Results of sample analysis of dosage form showed variability in 98.60–102.48% concentration range and the standard deviation was found to be between 0.0563–0.1291 while the variability of results for analyzing plasma samples was found to be between 98.8–101.11% and 91.32–99.61% concentration range for direct and indirect method and the standard deviation was found to be between 0.015–0.072 and 0.04–0.08 respectively. The limit of detection for direct procedure and indirect procedure was found to be 0.0194 and 0.175 μg/mL respectively. The method was validated according to ICH guidelines and values for accuracy, precision, specificity and robustness were found to be within the acceptable limits. The method was successfully employed for determination of concentration of etoricoxib in bulk drug, dosage form and for *in vitro* estimation of concentration of etoricoxib in human plasma.

Key Words: UV-Spectrophotometry, Etoricoxib.

INTRODUCTION

Etoricoxib, 5-chloro-6′-methyl-3[4-(methanesulfonyl) phenyl]-2,3′-bipyridine is a highly selective and active *cyclo*-oxygenase II inhibitor. This dipyridinyl derivative was developed in order to address the safety issues associated with traditional NSAIDs[1]. It is rapidly absorbed with the mean bioavailability of 100%. Etoricoxib reaches to maximum plasma concentration in approximately 1 h (T_{max}) after oral administration. Mean peak plasma concentration (C_{max}) of 3.6 mg/L

was reported after single oral dose of 120 mg. Peak plasma levels and AUC values are approximately linear with the dose. Etoricoxib has a volume of distribution of 120 L and is 92% plasma protein bound. In order to monitor intra- and inter-individual variability for the drug it is desirable to determine plasma concentrations of the drug in clinical practice. Literature review reveals few HPLC methods for estimation of etoricoxib in urine and plasma[2–4].

The present communication describes a simple and sensitive spectrophotometric method for the determination of concentration of etoricoxib in bulk drug, pharmaceutical dosage form and plasma with no complex separation or extraction steps involved[5–8].

EXPERIMENTAL

The present work was carried out on a Shimadzu UV-1700 series spectrophotometer, which possesses a double beam double detector configuration.

Reference standard of etoricoxib was a generous gift from Glenmark Pharmaceuticals Ltd., Ankleshwar, Gujarat. Methanol, sodium hydroxide, acetonitrile and ethyl acetate were of analytical grade, obtained from Merck Chemical Division, Mumbai. Plasma was procured from the local blood bank.

Standard drug solution containing etoricoxib 10 μg/mL was prepared using etoricoxib RS while plasma samples were prepared by spiking with known amount of etoricoxib stock solution in order to get standard spiked drug solutions containing etoricoxib (10 μg/mL). For indirect procedure, extraction of drug from plasma and for direct procedure precipitation of protein was done by employing optimized procedures described below and the solution was scanned over the range of 200–400 nm. Based on the spectral characteristics 280 nm was selected as the wavelength for analysis. This wavelength shows no interference to the selected solvent system and yields highly consistent results (Figs. 1 and 2).

Bulk drug and pharmaceutical dosage form: 10 mg of etoricoxib reference standard was accurately weighed and dissolved in 10 mg of 90% methanolic sodium hydroxide (0.1 M) to get Stock-A having concentration 1000 μg/mL. 1 mL of stock-A was diluted to get 100 μg/mL of stock-B) which was further diluted to get standard solutions in the range of 3–60 μg /mL.

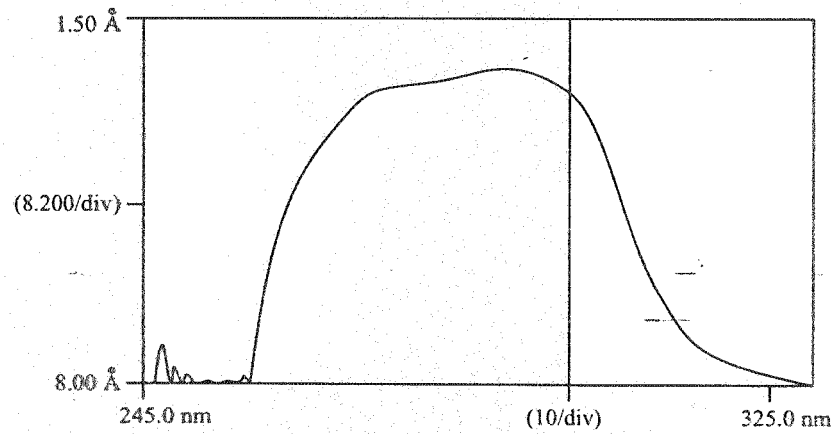

Fig. 1. Representative spectra of etoricoxib in acetonitrile precipitated solution.

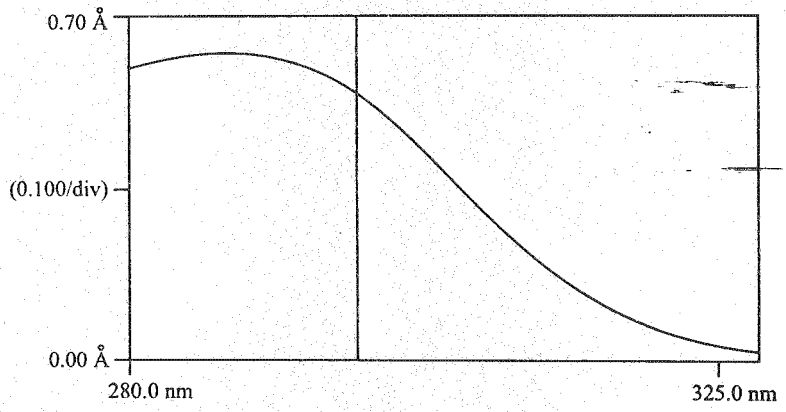

Fig. 2. Representative spectra of etoricoxib in ethyl acetate extract

Plasma samples: 10 mg of etoricoxib reference standard was accurately weighed and dissolved in 10 mg of methanol to get Stock-A having concentration 1000 μg/mL. 1 mL of stock-A was diluted with plasma to get 100 μg/mL Stock-B which was further diluted to get standard solutions in the range of 0.1–20 μg/mL.

Optimized procedure of sample preparation for plasma samples

Direct procedure: To 0.5 mL of plasma 9.5 mL of 21% sodium sulphite solution was added, mixed thoroughly and left to stand for 15 min at room temperature. After 15 min the solution was centrifuged at 3000 rpm for 10 min; the clear supernatant liquid was removed and analyzed.

Indirect procedure: To 4.0 mL of plasma in a stoppered separating funnel 5 mL of ethyl acetate was added, mixed thoroughly and left to stand for 5 min at room temperature. After 5 min the upper ethyl acetate layer was separated. The process was repeated twice with 3 mL and 2 mL ethyl acetate respectively. The ethyl acetate extracts were mixed and analyzed.

Quantitation: The standard curve was prepared in the respective solvent system by plotting absorbance against concentration using etoricoxib reference standard. By analyzing various laboratory samples applicability and reliability of the developed procedures was tested. For estimation of pharmaceutical formulations four marketed formulations of etoricoxib of various strengths were selected and sampled using statistical sampling techniques. The test samples of various concentrations in the linearity range were prepared from these selected formulations. Spiked plasma samples containing etoricoxib RS in concentrations ranging from 0.1–20 μg/mL were prepared and analyzed[9, 10].

Method Validation: The described procedures were validated for assay of component in bulk or in finished pharmaceutical formulations. To demonstrate the specificity of the method potential contaminants were generated using stress conditions like temperature and pH and analyzed. Linearity was studied by preparing standard solutions of different strengths. Accuracy was determined by recovery studies of etoricoxib. Precision was studied to find out inter- and

intra-day variations in the results of estimation while reproducibility was checked by repetitive analysis of the bulk drug and formulation using the developed procedures[11, 12].

Interference: By analyzing the solution subjected to stress conditions possible interference by various potential contaminants was tested. Interference by other drugs was also studied. Possible interference by normal plasma constituents was tested by scanning blank plasma treated in the way similar to that of spiked plasma.

Recovery: For the recovery study, to pre-analyzed tablet solution, a definite concentration of drug was added and then its recovery was studied. These studies were performed in two ways: one, by adding a fixed amount of pure drug solution to the final dilutions while varying the concentration of tablet sample solution in the final dilution and, second, by varying the amount of drug solution added to the final dilution keeping the concentration of sample solution in the final dilution constant and then calculating the recovery in both cases. For recovery study in plasma samples an exact volume of pure etoricoxib reference standard was prepared in acetonitrile and then analyzed. The absolute recovery was calculated by comparing the peak area with plasma standard. All the solutions were analyzed by the same procedure as for tablet analysis.

RESULTS AND DISCUSSION

Tablet samples: Based on the solubility, stability and spectral characteristics of the drug, 90% methanolic sodium hydroxide (0.1 N) was selected as the solvent system. Etoricoxib after being solubilized in the selected solvent was scanned in spectrum mode and 280 nm was selected as wavelength for estimation considering the reproducibility and variability of the obtained results.

The developed method is very simple, rapid and requires only accurate determination of the absorptivity of the drug at the selected wavelength. Though all the spectral recordings were done on Shimadzu UV-1700 series spectrophotometer, the method can be easily applied on other spectrophotometers.

The method was found to be linear in the range of 3–60 μg/mL and statistical analysis of results indicates good linearity (Table-1). The concentration of etoricoxib estimated in the tablet was found to be between 98.60–102.48% while the standard deviation values obtained from replicate analysis of tablet were found to be between 0.0563–0.1291 which indicates satisfactory applicability and reproducibility of the method (Table-2).

The results of recovery studies were also found to show variability in 98.25–100. 95% concentration range and standard deviation values were found to be 0.010–0.057 (Table-3). The generated potential contaminants do not show any significant interference in the spectrophotometric assay thus further confirming the applicability and reproducibility of the developed method.

TABLE-1
CALIBRATION CURVE DATA FOR SPECTROPHOTOMETRIC ANALYSIS OF ETORICOXIB

Conc. (μg/mL)	Replicate No.				
	1	2	3	4	5
	Absorbance				
3	0.104	0.105	0.106	0.103	0.107
4	0.138	0.139	0.138	0.133	0.139
5	0.177	0.179	0.179	0.177	0.184
10	0.361	0.363	0.364	0.363	0.381
20	0.744	0.747	0.745	0.751	0.790
30	1.110	1.118	1.115	1.114	1.167
40	1.453	1.454	1.460	1.468	1.560
50	1.833	1.835	1.840	1.847	1.949
60	2.201	2.203	2.209	2.218	2.341
Intercept	–0.0062	–0.0045	–0.0045	–0.0076	–0.0106
Slope	0.0368	0.0368	0.0369	0.0371	0.0392
R-value	0.9995	0.9997	0.9998	0.9998	0.9992

TABLE-2
ASSAY PRECISION OF SPECTROPHOTOMETRIC METHOD

Theoretical conc. (μg/mL)	Formulation	Abs. at 280 nm ±S.D. (n = 5)	Conc. found	% Found
12	I	0.449	12.290	102.410
	II	0.441	12.074	100.616
	III	0.439	12.020	100.160
	IV	0.447	12.237	101.975
16	I	0.600	16.383	102.393
	II	0.595	16.248	101.550
	III	0.598	16.329	102.056
	IV	0.591	16.139	100.868
20	I	0.751	20.475	102.375
	II	0.745	20.313	101.565
	III	0.749	20.421	102.105
	IV	0.741	20.204	101.020
30	I	1.128	30.692	102.306
	II	1.119	30.448	101.493
	III	1.125	30.611	102.036
	IV	1.121	30.502	101.673

TABLE-3
RESULTS OF RECOVERY STUDIES I AND II

Theoretical conc. (μg/mL)	Amount added (μg/mL)	Average conc. recovered ± S.D. (n = 5)	Recovery (%)
8	4	3.984	99.60
12	4	3.957	99.92
16	4	4.011	100.27
20	4	3.983	99.57
4	4	4.011	100.27
4	8	7.995	99.93
4	12	12.006	100.05
4	16	16.017	100.10

Plasma sample: The direct procedure was found to be linear in the range of 0.1–20 μg/mL (Table-4). The results of sample analysis showed variability in 98.8–101.11% concentration range while the standard deviation was found to be between 0.015–0.072, the limit of detection was found to be 0.0194 μg/mL.

The indirect procedure shows linearity in the range of 0.1–20 μg/mL (Table-4). Results showed variability in 91.32–99.61% concentration range while the standard deviation ranges between 0.04–0.08 and the limit of detection was determined as 0.175 μg/mL (Table-5). Both methods were statistically validated.

TABLE-4
CALIBRATION CURVE AND ASSAY PRECISION DATA FOR THE ANALYSIS OF ETORICOXIB IN PLASMA SAMPLES

Conc. (μg/mL)	Direct method Absorbance at 280.0 nm ± S.D. (n = 3)	Indirect method Absorbance at 280 nm ± S.D. (n = 3)
0.0	0.000 ± 0.000	0.000 ± 0.000
2.5	0.266 ± 0.015	0.138 ± 0.001
5.0	0.513 ± 0.011	0.272 ± 0.005
10.0	1.008 ± 0.012	0.571 ± 0.012
15.0	1.502 ± 0.004	0.805 ± 0.004
20.0	1.997 ± 0.008	1.076 ± 0.017
Slope	0.0989	0.0536
Intercept	0.0190	0.0056
R^2	0.9998	0.9994

TABLE-5
DATA FOR LIMIT OF DETECTION IN PLASMA SAMPLES

Conc. (μg/mL)	Direct method Absorbance at 280 nm ± S.D. (n = 3)	Indirect method Absorbance at 280 nm ± S.D. (n = 3)
1	0.107 ± 0.004	0.042 ± 0.001
2	0.215 ± 0.001	0.091 ± 0.003
3	0.322 ± 0.006	0.122 ± 0.007
4	0.430 ± 0.008	0.175 ± 0.001
5	0.537 ± 0.003	0.227 ± 0.005

Conclusion

All these parameters suggest the method is precise, accurate and reproducible and hence the developed procedure can be used for the estimation of etoricoxib in bulk drug, pharmaceutical dosage form and plasma samples.

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