Chapter V

Analytical monitoring of Diclofenac sodium
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Simple, suitable cost effective analytical method is required for estimation of diclofenac sodium in pharmaceutical samples and formulations. Among different methods available in the pharmacopoeia, spectrophotometric method is selected for routine analysis. *In vivo* estimation of diclofenac sodium in rabbit plasma is done by HPLC method.

1. **Preparation of 0.1 (N) HCl solution**

8.5 ml of concentrated hydrochloric acid was taken in a volumetric flask containing 200 ml distilled water and the volume was then made upto 1000 ml with distilled water and finally pH was adjusted to 1.2 using a pH meter.

2. **Preparation of USP Phosphate buffer of pH 6.8**

6.808 gm of potassium di-hydrogen phosphate (KH$_2$PO$_4$), accurately weighed, was dissolved in 900 ml distilled water, 0.896 gm of NaOH was dissolved in 25 ml distilled water in a beaker and mixed with the above solution. Then the pH was adjusted to 6.8 using pH meter by slowly adding NaOH solution and finally the volume was made up to 1000 ml with distilled water.

3. **Determination of maximum wavelength ($\lambda_{\text{max}}$) of diclofenac sodium in pH 1.2**

The maximum wavelength ($\lambda_{\text{max}}$) was determined in pH 1.2 HCl buffer. 10 mg of diclofenac sodium was weighed accurately and was dissolved in 100 ml volumetric flask and the volume was made upto 100 ml by using 0.1 (N) HCl buffer of pH 1.2 to get the concentration of 100 µg/ml of standard diclofenac sodium. Thus the stock solution of
standard diclofenac sodium was prepared. The scanning of the stock solution was performed by using a UV spectrophotometer (Thermo Scientific, Evolution 201). The maximum wavelength ($\lambda_{max}$) was found to be at 276 nm (Fig 1).

4. Preparation of standard curve of diclofenac sodium in pH 1.2

From the above prepared stock solution, five dilutions were made by using 0.1 (N) HCl buffer of pH 1.2 which has ultimately the concentrations of 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml, and 5 µg/ml. The absorbance of the dilute solutions was measured by UV spectrophotometer at 276 nm. The standard curve of diclofenac sodium was prepared by plotting absorbances on Y-axis against concentration on X-axis. The slope of the standard curve was obtained (Fig 3).

5. Determination of maximum wavelength ($\lambda_{max}$) of Diclofenac sodium in phosphate buffer of pH 6.8

The maximum wavelength ($\lambda_{max}$) of Diclofenac sodium was determined in pH 6.8 phosphate buffer. 10 mg of Diclofenac sodium was weighed accurately and was dissolved in 100 ml volumetric flask and the volume was made upto 100 ml by using 0.2 (M) phosphate buffer of pH 6.8 to get the concentration of 100 µg/ml of standard Diclofenac sodium. Thus the stock solution of standard Diclofenac sodium was prepared. The scanning of the stock solution was performed by using a UV spectrophotometer. The maximum wavelength ($\lambda_{max}$) was found to be at 276 nm (Fig 2).
6. Preparation of standard curve of Diclofenac sodium in phosphate buffer of pH 6.8

From the above prepared stock solution, five dilutions were made by using 0.2 (M) phosphate buffer of pH 6.8 which has ultimately the concentrations of 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml, and 5 µg/ml. The absorbance of the dilute solutions was measured by UV spectrophotometer at 276 nm. The standard curve of Diclofenac sodium was prepared by plotting absorbances on Y-axis against concentrations on X-axis. The slope of the standard curve was obtained (Fig 4).

7. Estimation of diclofenac sodium in rabbit plasma

In the present study, plasma Diclofenac Sodium concentration in rabbits was determined by the HPLC method adopted by Giagoudakis and Markantonis (1998). An isocratic HPLC (Merck) with L-7400 UV detector, L7110 isocratic pump, Kromasil 250-4.6 HPLC column, rheodyne injector was used. The degassed mobile phase consisted of acetonitrile–0.1 M sodium acetate (35:65v/v) adjusted to pH 6.3 with glacial acetic acid. The filtered mobile phase was pumped from the reservoir at a flow rate of 0.9 ml/min. The eluent was monitored by a UV detector at 278 nm, and the data were acquired, stored, and analyzed.

For the preparation of standard curve in rabbit plasma, first stock solution of drug of concentration 100 ngm/ml was prepared. From stock solution, four different concentrations of diclofenac sodium in rabbit plasma (50 ngm/ml, 100 ngm/ml, 200 ngm/ml, 300 ngm/ml) were prepared by adding the required volume of stock solution in standard serum sample. 0.5 ml serum from the previous samples were extracted separately with 5 ml acetonitrile (HPLC grade). Then it was shaken for 15 mins. Then the mixture was centrifuged at 2000 rpm for 10 mins. The supernatant liquid was evaporated to dryness under nitrogen atmosphere at 37 degree centigrade. This method was followed by dissolving the residue after extraction in 2 ml mobile phase and subjected to HPLC analysis. Calibration curve was prepared by taking area on y axis.
and concentrations of drug (ng/ml) on x axis. HPLC chromatogram of Diclofenac sodium is shown in Fig 5.
Fig 1. Scanning report of pure diclofenac sodium showing a sharp wavelength at 276 nm in pH-1.2.
Fig 2: Scanning report of pure diclofenac sodium showing a sharp wavelength at 276 nm in phosphate buffer solution of pH-6.8.
Figure 3: Standard curve of diclofenac sodium in pH-1.2

\[ y = 0.027x \]

\[ R^2 = 0.996 \]
Fig 4. Standard curve of diclofenac sodium in phosphate buffer of pH 6.8
Fig 5: HPLC chromatogram of diclofenac sodium in plasma
Discussion

The spectrum of diclofenac sodium in phosphate buffer of pH 6.8 (Fig 2) and HCl solution of pH 1.2 (Fig 1) showed a distinct $\lambda_{\text{max}}$ at 276 nm. The absorbance at 276 nm was found to be stable for at least 24 hr at 25±2°C indicating stability of the drug in the selected media. At all concentration levels the SD was low and the relative standard deviation did not exceed 0.03. The linear regression equation was obtained with excellent regression coefficient.

During HPLC analysis, a good linear relationship was observed between the concentration of diclofenac sodium and the peak area of drug with a high correlation coefficient ($r^2 = 0.9998$).
Reference