Chapter IV

Methodology

Analytical monitoring of Tramadol Hydrochloride
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Analysis is an important and integral component in the formulation development of any drug molecule and characterization of the developed formulations. Suitable and validated analytical methods are imperative for estimation of the drug in a variety of samples like bulk powders, formulations, in vitro release samples. Different analytical methods have been reported in the pharmacopoeias for estimation of Tramadol hydrochloride and HPLC method is the most precise one, but use of HPLC methods for the routine analysis of drug samples specially from in vitro release studies of sustained release formulations, involving multiple time points seems to be cumbersome, costly and time consuming. For routine in vitro estimation of drug, spectrophotometric method was used.

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 the 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 (KH₂PO₄), 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 tramadol HCl in 0.1 (N) hydrochloric acid buffer of pH 1.2

The maximum wavelength (\(\lambda_{\text{max}}\)) of tramadol HCl was determined using pH 1.2 HCl buffer. 10 mg of tramadol HCl was weighed accurately and was dissolved in 100 ml volumetric flask and the volume was made up to 100 ml by using 0.1 (N) HCl buffer of pH 1.2 to get the concentration of 100 µg/ml of standard tramadol HCl. Thus the stock solution of standard tramadol HCl was prepared. The scanning of the stock solution was performed by using a UV spectrophotometer (Thermo Scientific, Evolution 201). The maximum wavelength (\(\lambda_{\text{max}}\)) was found to be at 271 nm.

4. Preparation of standard curve of tramadol HCl in 0.1 (N) hydrochloric acid buffer of 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 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml. The absorbances of the dilute solutions were measured by UV spectrophotometer at 271 nm. The standard curve of tramadol HCl was prepared by plotting absorbances on Y- axis against concentrations on X- axis. The slope of the standard curve was obtained.
5. Determination of maximum wavelength ($\lambda_{\text{max}}$) of tramadol HCl in phosphate buffer of pH 6.8

The maximum wavelength ($\lambda_{\text{max}}$) of tramadol HCl was determined in pH 6.8 phosphate buffer. 10 mg of tramadol HCl 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 tramadol HCl. Thus the stock solution of standard tramadol HCl was prepared. The scanning of the stock solution was performed by using a UV spectrophotometer. The maximum wavelength ($\lambda_{\text{max}}$) was found to be at 271 nm.

6. Preparation of standard curve of tramadol HCl 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 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml. The absorbances of the dilute solutions were measured by UV spectrophotometer at 271 nm. The standard curve of tramadol HCl was prepared by plotting absorbances on Y- axis against concentration on X- axis. The slope of the standard curve was obtained.
Results and discussion

Fig 1: Scanning report of pure tramadol HCl showing maximum wavelength $\lambda_{max}$ at 271 nm 0.1 (N) hydrochloric acid buffer.

Fig 2: Scanning report of pure tramadol HCl showing maximum wavelength $\lambda_{max}$ at 271 nm in phosphate buffer at pH 6.8.
Fig 3: Standard curve of tramadol hydrochloride in pH 1.2
Fig 4: Standard curve of tramadol hydrochloride in pH 6.8
Discussion

The spectrum of tramadol hydrochloride in phosphate buffer of pH 6.8 and HCl solution of pH 1.2 showed a distinct $\lambda_{\text{max}}$ at 271 nm. The absorbance at 271 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. The linear regression equation was obtained at pH 1.2 and pH 6.8 with an excellent regression coefficient.