ABSTRACT

Tramadol HCl is often used as an analgesic as mono-therapy as well as in combination with other analgesic for treating severe and unavoidable pain in patients. Tramadol HCl acts on serotonergic and noradrenergic nociception and being a "PSYCHOTROPIC SUBSTANCE" its long term use can cause addiction in patients. Different formulation are being synthesized and used for this centrally acting analgesic of opioid category. Pharmaceutical companies always look for a simple and efficient method for qualitative and quantitative analysis of these formulations in their quality control and quality assurance laboratories. As Tramadol HCl administration has a risk of addiction due to misuse and abuse of this drug, a fast and accurate method is required for forensic investigation as well. Therefore, a simple, efficient, accurate and fast RP-HPLC (**Reverse Phase High Pressure Liquid Chromatography**) method was developed by optimizing chromatographic conditions for injection volume 20µL, 0.8ml/min mobile phase flow rate, 10 min run time, column temperature 25 °C and detection was performed in Isocratic mode at preferred wavelength 220nm by diode array detector. Mobile phase used was consisted of methanol: phosphate buffer (20:80 v/v) and its pH was adjusted at 2.4 with the help of phosphoric acid. Under these conditions well resolved Tramadol HCl peaks were obtained and the method was further validated by using ICH guidelines by spiking 02 µg/mL, 50 µg/mL, and 80 µg/mL in human plasma. Dichloromethane and Iso-propane were used to deproteinize human plasma. Deproteinized supernatant was separated and injected in HPLC system. The average retention time was no more than 4 minutes. Regression analysis over the range of 1-250 µg/mL demonstrated efficient correlation with regards to $R^2 \geq 0.996$. Method accuracy was analyzed in term of recovery and average recovery was 94.5-97.80%. Inter-day and intraday precision obtained in term of percentage deviation were 2.2-3.5 and 2.38-5.5, respectively. LOD and LOQ of this method was 0.2 µg/ml and 1.8 µg/ml, respectively. The proposed HPLC method has short analysis time and is robust, 2

specific, efficient and appropriate for the Tramadol HCl determination in biological samples such as blood and can be used in forensic investigation for pharmacokinetic testing of drug.