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DEVELOP A SIMPLE AND ROBUST REVERSED-PHASE HPLC TECHNIQUE FOR SIMULTANEOUS DETERMINATION OF TRAMADOL IN PLASMA

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Keywords:

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ABSTRACT

The optimized method for HPLC determination of tramadol in plasma has been developed and validated by a novel approach.The chromatographic analysis was performed at ambient temperature with a chromatographic system used was Shimadzu LC-2010separation, dual wavelength absorbance detector SPD-20A detector equipped with Spinchrom LC solution software,C18 (150 × 4.6 mm) 5.0 micron, maintained at 25° C, eluted with mobile phase at the flow rate of 1.0 ml/min. The mobile phase consisted of acetonitrile: phosphate buffer 0.01 M (30:70) pH adjusted to 3.5 with ortho phosphoric acid, 0.1 % triethylamine (TEA) was added to reduce asymmetry and retention time. Sample volume of 20 µl was injected into the HPLC column and elute was monitored at 220 nm.The high correlation coefficient (r^2> 0.997) values indicated clear correlations between the investigated compound concentrations and their peak areas within the test ranges. The repeatability and intermediate precision, expressed by the RSD, were less than 2%. The accuracy evaluated by performing recovery studies via a spike method, was in the range. From the results of tramadol hydrochloride analysis it can be concluded that the proposed HPLC method is precise, linear and robust that can be used for routine analysis.

1. INTRODUCTION

Tramadol hydrochloride is a m-receptor agonist used in the treatment of mild to moderate pain. (1RS, 2RS)-2-[(Dimethylamino) methyl]-1-(3- methoxyphenyl)cyclohexanol hydrochloride (Fig. no 1). [1] Its therapeutic concentration is in the range 100–300 ng/ml [2]. After a single bolus infusion of 100 mg tramadol, concentrations in plasma can be detected instantaneously. Elimination is slow, being characterised by an elimination half-life of 6 h [2]. It has been used since 1977 for the relief of strong physical pain and has been the most widely sold opioid analgesic drug in the world [3]. The tramadol was determined by HPLC with UV detection [4–6], fluorescence detection or electrochemical detection [7-8]. Capillary gas chromatography was also media in literature. The present work is to develop a simple and robust reversed-phase HPLC technique for simultaneous determination of tramadol in human plasma by using UV-Vis Spectrophotometer and HPLC.

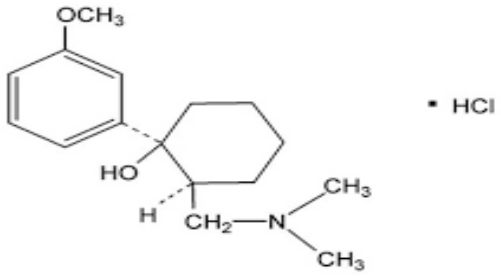

Fig no. 1

2. EXPERIMENTAL

2.1 Chemicals and reagents

Tramadol standard and drug sample was provided as a gift sample from Wockhardt Ltd. India. Water, acetonitrile, chloroform and methanol used were of HPLC grade (Merck, India). BufferSolution (potassium dihydrogen phosphate, KH2PO4) was prepared with deionised water.

2.2 *Instrumentation*

Shimadzu LC-2010separation, dual wavelength absorbance detector SPD-20A detector equipped with Spinchrom LC solution software,C18 (150 × 4.6 mm) 5.0 micron

2.3 *Chromatographic conditions*

The chromatographic analysis was performed at ambient temperature with a chromatographic system used was Shimadzu LC-2010separation, dual wavelength absorbance detector SPD-20A detector equipped with Spinchrom LC solution software,C18 (150 × 4.6 mm) 5.0 micron, maintained at 25° C, eluted with mobile phase at the flow rate of 1.0 ml/min. The

mobile phase consisted of acetonitrile: phosphate buffer 0.01 M (30:70) pH adjusted to 3.5 with ortho phosphoric acid, 0.1 % triethylamine (TEA) was added to reduce asymmetry and retention time. Sample volume of 20 µl was injected into the HPLC column and elute was monitored at 220 nm.The mobile phase filtered through 0. 45 µm nylon membranes filter and degassed in ultrasonic bath prior to use.

2.4 System Suitability:-

2.4.1 Selection of system suitability solution

The resolution tramadol hydrochloride was considered and other factors which were included were tailing factor, theoretical plates and %RSD (6 injections) based on diluted standard of the standard solution.

2.4.12Preparation of system suitability solution

Weight accurately about 10 mg of tramadol hydrochloride reference/working standard into a 100 ml volumetric flask. Dissolve and dilute to volume with diluent. Into a separate 50 ml volumetric flask, weigh accurately about 23 mg of pantoprazole sodium reference/working standard. Dissolve in about 10 ml of diluents. Add 1.0 ml of Imp Stock Sol. into this flask and diluted to volume with diluent.

2.4.3 Preparation of sample solution

Sample solutions of tramadol hydrochloride were prepared by dissolving 10 mg appropriate amounts of 100 ml in mobile phase to obtain final drug concentration100µg/ml.

2.5 Validation Parameters

2.5.1 Accuracy and Precision

System suitability solution was prepared as given above. Blank, system suitability solution was injected as per injection sequence and the acceptance criteria for system suitability was checked. Blank and six replicate injections of system suitability solution were injected. Resolution was checked between tramadol hydrochloride peaks and % RSD of peak areas was calculated for tramadol hydrochloride in the chromatogram obtained with six replicates of system suitability solution.

2.5.3Linearity and Range

For the calibration standards, dilutions 0.01, 0.025, 0.050, 0.075, 0.1, and 0.15µg/mlwere prepared from stock solutions. The dilutions were prepared and portion of this solution was filtered through 0.45 µma disposable membrane filter and then injected to HPLC

2.5.4Limit of detection

The limit of detection (LOD) of the drug was calculated by using the following equations designated by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3 \times \sigma/S,$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

2.5.5Limit of quantification

The limit of quantification (LOQ) of the drug was calculated by using the following equations designated by International Conference on Harmonization (ICH) guideline:

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

3. RESULT AND DISCUSSION

Optimization of chromatographic conditions

Data from six injections of system suitability solution were utilized for calculating parameters for system suitability. The resolution of tramadol hydrochloride was resolved. Figure 1. It showed that the proposed method is precise. Hence, it can be concluded that the system suitability parameter meets the requirement of method validation. Peak was observed to be well resolved and Retention time is shown in Table 1

Table 1 Chromatographic Conditions for final optimized method

Column	Hypersil ODS(125X4.0)mm,5 µm
Column Temperature	25° C
Flow Rate	1.0 mL per minute
Gradient Program	Acetonitrile: Phosphate buffer (30:70)
Injection Volume	20 µL
Detector wavelength	220 nm
Run Time	4 min
Retention time	2.5
%RSD	7917

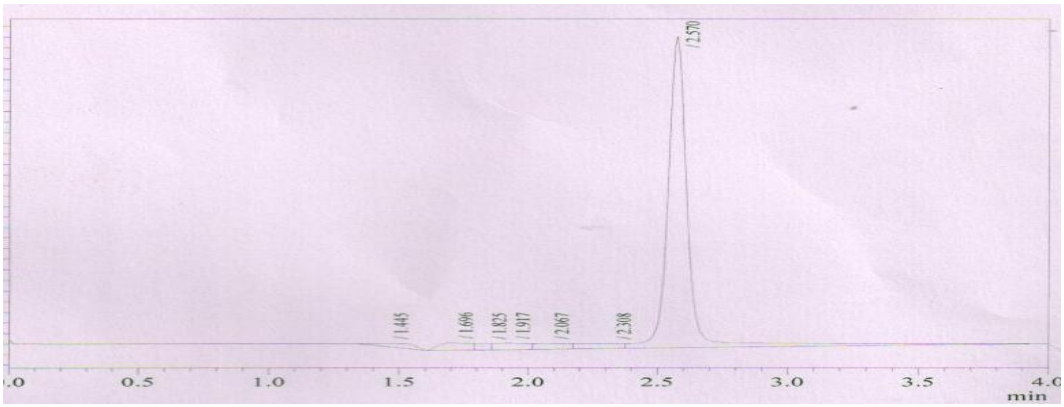

Figure 2 Typical representative Chromatogram tramadol hydrochloride control

Accuracy and precision

Accuracy andprecision of the test was evaluated by determining the inter-day and intra-day relative standarddeviation (RSD) of the measured peak arearatios for different concentrations. It wasexpressed as the mean, SD, percentage RSD of analyte reportedin the Table 2-3.

Table2. Study of Precision and accuracy Parameter by RP-HPLC method for the determination of tramadol in plasma

Method	Parameters	System Precision	Method Precision	Intermediate Precision	
				Inter-day	Intraday
RP-HPLC	Mean	7860	7153	7841	7844
	± SD	92.6	57.98276	28.28	45.25
	% RSD	1.2	0.81	0.3	0.5

Table 3. Percent recoveries in commercial formulations by RP-HPLC methods of analysis

RP-HPLC		
Mean	% RSD	% Recovery
6305	0.31	98.39
5675	0.06	99.04
7487	0.14	99.48

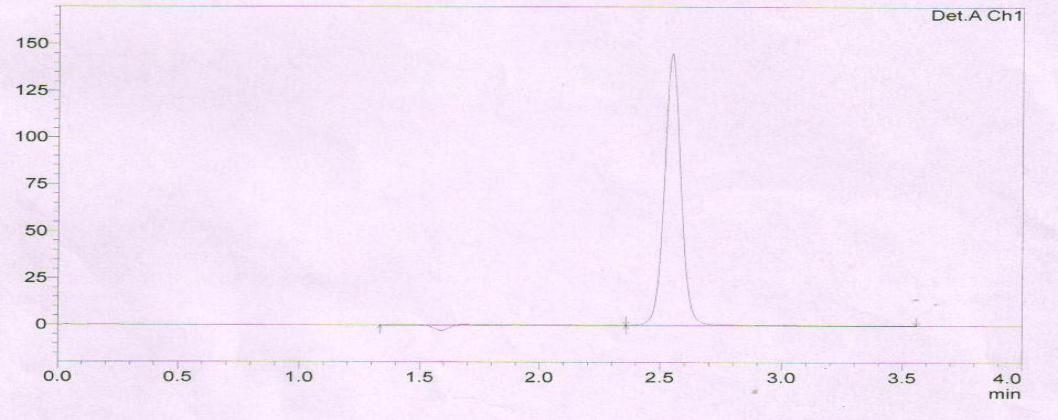

Figure 3 .Typical representativeChromatogram tramadol hydrochloride for formulation

Calibration Curve

Linearity range for the tramadol hydrochloride was obtained in the concentration range 0.010 to 0.150 µg/ml. It was further confirmed by regression analysis R^2.Figure 4 and Table 4.

Table: 4 Standard curve of tramadol hydrochloride in plasma by HPLC method

S.No.	Concentration (µg/ml)	Peak Area
1	0.010	926
2	0.025	2018
3	0.050	3581
4	0.075	5485
5	0.100	7782
6	0.150	11274
Equation of linearity	y = 74744x + 70.134	
Coefficient R^2	0.997	

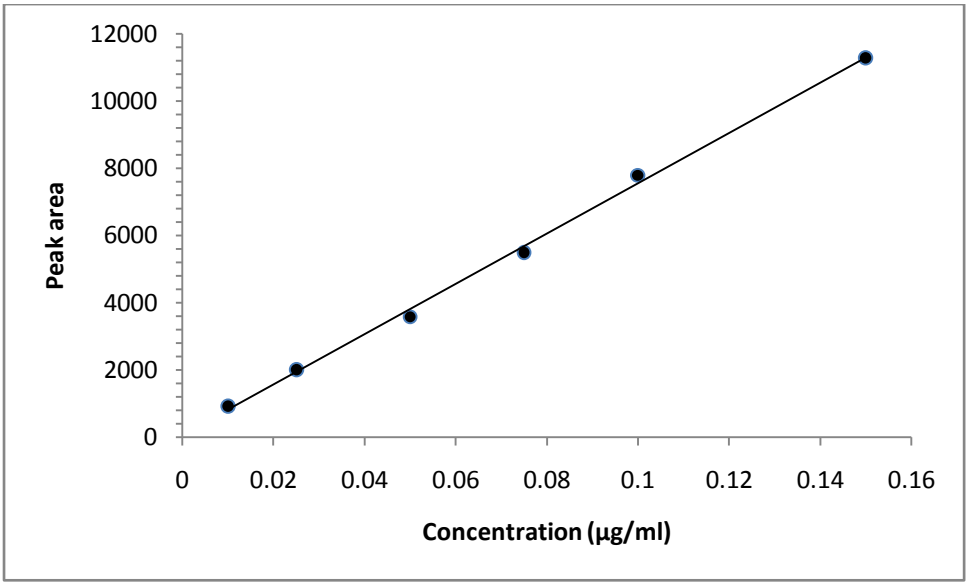

Figure 4Calibration curve for tramadol hydrochloride in plasma

Limit of detection

The limit of detection (LOD) ofthetramadol hydrochloride was calculated by using equationsof0.005µg/ml. So the lowest level of concentration can be detected by method andwas found as 1.7.

Limit of quantification

The limit of quantitation (LOQ) ofthetramadol hydrochloride was calculated by using equationsof0.01µg/ml.So the lowest level of concentration can be quantified by method and was found as 5.1.

4. CONCLUSION

The optimized method for HPLC determination of tramadol in plasma has been developed and validated by a novel approach. The applied methods are advantageous in having simple and rapid for the determination of the concentration of tramadol hydrochloride when compared with other methods in the literature for the routine determination. In particular, the method has satisfactory specificity, linearity, accuracy and precision range over the concentration range examined.

5. ACKNOWLEDGEMENTS

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