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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF TRAMADOL IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A validated simple, sensitive, specific and precise RP-HPLC method was developed for the determination of Tramadol in pure and pharmaceutical formulations. Method was carried on Zodiac C_{18} column (250 mm × 4.6 mm × 5 µ particle size) using Methanol: Acetonitrile: water (15 : 60 : 25) as mobile phase. Detection was carried out by U.V. at 218 nm. The proposed method obeyed linearity in the range of 60-200 µg/mL and met all specifications as per ICH guidelines. Statistical analysis revealed that this method can be used in routine quality control studies of Tramadol in pure and its formulations.

Key words: Tramadol, C_{18} column, Reverse phase, Validation, Spectificity.

INTRODUCTION

Tramadol hydrochloride chemically (±) Cis-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl)- cyclohexanol) is a synthetic, centrally acting, analgesic agent, used for the relief of moderate to chronic pain and has no clinically relevant cardiovascular or respiratory depressant activity[1]. Tramadol is official in B.P[2]. A literature review reveals that only a few methods have been developed for the quantification of individual drug Tramadol as by HPLC[3,4] determination of tramadol in human plasma and urine by HPLC[5], HPLC method for tramadol in human plasma using liquid–liquid extraction[6], HPLC and enantioselective HPLC method for tramadol and o-desmethyl tramadol determination in human plasma and urine[7,8]. In the present study, a new RP-HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines[9,10].

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Instrumentation

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Tramadol an isocratic PEAK HPLC instrument with Zodiac C_{18} column (250 mm x 4.6 mm, 5 µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC 7000 UV-detector. A 20 µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Standards and chemicals used

Tramadol was provided by Dr. Reddy's laboratories. All the chemicals Acetonitrile, methanol, water were HPLC grade, Merck Specialties Private Limited, Mumbai, India.

Commercial tablets of Tramadol were purchased from local market.

Preparation of the mobile phase

Into a 1000 mL cleaned volumetric flask, HPLC grade methanol 150 mL, acetonitrile 600 mL and water 250 mL (which are filtered through 0.25 mm membrane filters by vacuum filtration) were slowly added, mixed well and sonicated upto 20 min. Cool the above solution and pH was adjusted to 4.2 with orthophosphoric acid. This solution is again sonicated to 10 min. Cool the solution to room temperature and use for chromatography method.

Preparation of standard drug solutions

100 mg of Tramadol was accurately weighed and is dissolved in few mL of the mobile phase and sonicated for few min to dissolve the drug completely. Then it is filtered through 0.2 µ ultipore filter paper and the volume is made upto 100 mL with mobile phase to get a concentration of 1 mg/mL (free base) stock solution. This solution is further diluted with same solvent to obtain required working standard concentrations.

Sample preparation

20 commercial tablets of Tramadol were finely powdered and the powder equivalent to 10 mg of Tramadol accurately weighed to 50 mL volumetric flask and dissolved in few mL of mobile phase. The above solution was subjected to sonication for 15 min. After getting clear solution it is filtered through 0.25 µm membrane filters and the solution is made upto 50 mL with mobile phase resulting in preparation of 1 mg/mL solution. This is further diluted so as to obtain required concentration of Tramadol pharmaceutical dosage form.

Methodology

The HPLC system was stabilized for thirty min. by passing mobile phase, detector was set at 211 nm, flow rate of 1.0 mL/min to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Eight concentrations of each standard solutions 60, 80, 100, 120, 140, 160, 180, 200 µg/mL were injected. Calibration graph was plotting concentration on X-axis and peak areas on Y-axis. The amount of drug present in sample was computed in calibration graph.

Pharmaceutical formulations

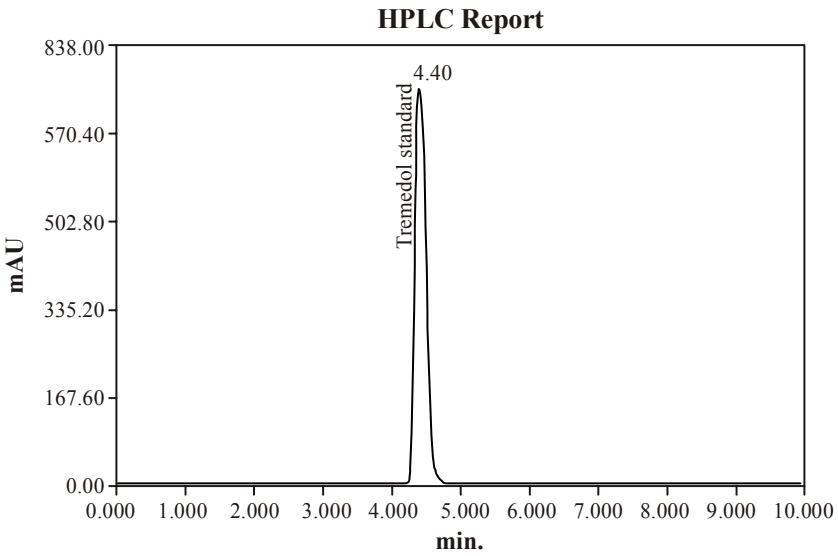
Prepared dilution of pharmaceutical formulation is injected and the procedure described under bulk samples was followed. The amount of drug present in sample was computed in calibration graph.

Table 1: Optimized chromatographic conditions for estimation of Tramadol

Parameter	Condition
Mobile phase	Methanol : ACN : water (15 : 60 : 25) (v/v/v)
Pump mode	Isocratic
pH	4.2
Diluents	Mobile phase
Column	Zodiac C_{18} column (250 x 4.6 mm, 5 µ)
Column Temp	Ambient
Wavelength	218 nm
Injection Volume	20 µL
Flow rate	1.0 mL/min
Run time	10 min
Retention Time	4.40 min

RESULTS AND DISCUSSION

The objective of the present work is to develop simple, precise and reliable HPLC method for the analysis of Tramadol in bulk and pharmaceutical dosage form. This is achieved by using the most commonly employed column C_{18} with U.V. detection at 218 nm. The representative chromatogram indicating Tramadol is shown in Fig. 1.



ID	Name	Retain T.	Height	Area	Conc.	Tail. factor	Theo. plate
1	Tremedol standard	4.395	75979	869455.1	100.000	1.09	2940
	Sum:		75979	869455.1	100.0000		

Fig. 1: Chromatogram of Tramadol

Parameter fixation

In developing this method, a systemic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

Stationary phase characteristics

Based on nature and solubility characteristics of Tramadol, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C_{18} column was found to be optimum.

Mobile phase characteristics

In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of Methanol : ACN : water (15 : 60 : 25)

(v/v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Validation of the proposed method

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

Linearity

It is the ability of the method to elicit test results directly propotional to analyte concentration with in a given range[11]. Linearity was performed by preparing standard solutions of Tramadol at different concentration levels, twenty micro liters of each concentration was injected in into the HPLC system. The peak responses were read at 218 nm and the corresponding chromatograms were recorded. Linearity plots of concentration over peak areas was constructed. Linearity results were obtained in the concentration range of 60-200 µg/mL. The results were presented in Table 2.

Table 2: Linearity results of tramadol

Concentration (µg/mL)	Peak area
60	386922
80	521828
100	627273
120	757245
140	869455
160	993104
180	1106645
200	1223336

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intra day precision, inter day precision.

Intra day precision

To study the intra day precision, six replicate standard solutions (140 µg/mL) of Tramadol was injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.15, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 3.

Table 3: Intra day precision values

Sample	Conc. (in ppm)	Injection No.	Peak areas	RSD (Acceptance criteria ≤ 2.0%)
Gabapentin	140	1	869455	0.15
		2	866878	
		3	866270	
		4	868997	
		5	869182	
		6	868510	

Inter day precision

To study the interday precision, six replicate standard solutions (140 ppm) of Gabapentin was injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.09, which are well within the acceptable criteria of not more than 2.0.

Specificity

The effect of wide range of excipients and other additives usually present in the formulation of Tramadol in the determinations under optimum conditions were investigated infact, may have no observation at this UV Maximum. Chromatographic parameters maintained are specific for Tramadol.

Ruggedness

Percent recoveries of Tramadol was good under most conditions and didn't show any significant change, when the critical parameters were modified. An ICH choose instead to cover the topic of ruggedness as part of Precision (reproducibility)[12].

Table 4: Ruggedness values

Sample	Conc. (in ppm)	Injection No.	Peak areas	RSD (Acceptance criteria ≤ 2.0%)
Tramadol	140	1	870252	0.15
		2	868003	
		3	866833	
		4	867928	
		5	869409	
		6	866914	

Table 5: Limit of detection and limit of quantification for Tramadol

Parameter	Values
Limit of quantification	1.8 µg/mL
Limit of detection	0.6 µg/mL

Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed Standard solution. The standard addition method was performed at 50%, 100% and 150% level of 80 ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table 6. Satisfactory recoveries ranging from 99% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate.

Table 6: Recovery results

Level	Concentration (µg/mL)	Amount of Tramadol spiked (µg/mL)	Total in ppm	Amount of Tramadol recovered (ppm)	% Recovery
50 %	80	40	120	119.7	99.78
	80	40	120	119.4	99.6
	80	40	120	119.8	99.9

Cont…

Level	Concentration (µg/mL)	Amount of Tramadol spiked (µg/mL)	Total in ppm	Amount of Tramadol recovered (ppm)	% Recovery
100%	80	80	160	159.1	99.5
	80	80	160	161.3	100.9
	80	80	160	162.6	101.7
150%	80	120	200	199.1	99.6
	80	120	200	201.54	100.77
	80	120	200	200.20	100.1

Robustness

The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. Tramadol at 2 ppm concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.

Formulation

S. No.	Brand Name	Concentration	Area	Amount found	% Assay
1	Bestodol-50 mg	140 ppm	867938	138.97	99.26

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