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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF TRAMADOL IN EXTENDED RELEASE TABLET PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Tramadol is a synthetic codeine analogue, which has analgesic properties with effects similar to opioids such as morphine and codeine. A simple, selective, precise, accurate and cost effective reverse phase HPLC method has been developed and validated for estimation of Tramadol in extended release tablet dosage form. In the chromatographic conditions, Zorbax C18 (150 X 4.6 mm, 5µ) stationary phase with mobile phase consisting of 5mM ammonium acetate buffer (pH 4.0 ± 0.3): acetonitrile (15: 85 v/v) was used at a flow rate of 0.8 mL/min. and column temperature was maintained at 40°C. Tramadol was detected at 270 nm. The chromatographic procedure separated Tramadol and potential interfering peaks in an analysis time of 2.5 min. with Tramadol eluting at about 1.6 min. The assay method was found linear in the concentration range of 0.008 to 0.500 mg/mL with a correlation coefficient of 0.9998. The percentage recovery of assay was found between 98.5 and 100.8. The developed method was validated with respect to specificity, linearity, accuracy, precision, sensitivity, robustness and solution stability as per ICH guidelines. The proposed method can be used for routine analysis of Tramadol formulations in quality control laboratories.

KEY WORDS

Tramadol, HPLC, Validation, Dissolution, Extended Release

INTRODUCTION:

Tramadol is an opioid pain medication used to treat moderate to severe pain[1]. When taken orally as an immediate release formulation, the onset of pain relief usually occurs within an hour.[2] It is often combined with acetaminophen to improve the efficacy of tramadol in relieving pain.[3] Tramadol undergoes demethylation to form O-desmethyl metabolite (M1). Both tramadol and its O-desmethyl metabolite are selective, weak OP3-receptor agonists.

Reverse Phase HPLC: In this chromatographic technique, the stationary phase is non-polar, and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first.[3]

Steps involved in development of RP-HPLC method:

Selection of chromatographic method: The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drug selected is polar and ionic hence Reverse phase chromatography was used because of its simplicity and suitability.[4]

Selection of stationary phase: Matching the polarity of sample and stationary phase and using a mobile phase of different polarity will achieve a successful separation.[5]

Selection of mobile phase: Reverse phase bonded packing, when used in conjunction with highly polar solvents; approach is ideal and is a universal system for liquid chromatography. Mobile phase may be either single liquid or combination of liquids, which are compatible with sample, column and instrument.[6]

Selection of suitable detector: Detector is the eye of HPLC system that measures the compounds after their separation on the column. There are basically two types of detectors- the bulk property detectors and solute property detectors. Detectors, in order of their popularity are UV, fluorescent, conductivity, polarimeter and refractive index detectors. UV detector is the first choice because of its convenience and applicability in case of most of the samples. The latest versions of equipment's are available with photo diode-array detectors (PAD or DAD).

Method optimization:

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and shape, plate counts asymmetry, capacity, elution time, detection limits, limit of quantitation, and overall ability to quantify the specific analyte of interest. Literature search reveals, several analytical methods were reported for the determination of Tramadol alone or in combination with other drugs in active pharmaceutical ingredients, oral and parenteral formulations. These methods used different detection techniques like spectrophotometry[4-8], HPTLC[9-10] and HPLC[11-18]. In the current work we have made an attempt to develop simple, robust, cost effective and high throughput analytical method for the determination of Tramadol in tablet dosage form. The method uses UV detection with a run time of 2.5 min. The method has several advantages like simple mobile phase, low injection volume, less run time over the reported methods. The developed method was validated as per international conference on harmonization (ICH) Q2 (R2) guidelines.[9]

MATERIALS AND METHODS

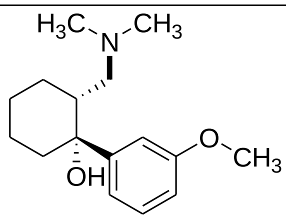

Fig.1.Structure of Tramadol HCl

Drug profile of Tramadol

IUPAC Name:(±) cis-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride.

Chemical formula: $C_{16}H_{25}NO_2$ .HCl

Molecular weight: 299.84

Description: White or almost White crystalline powder.

Solubility: Freely soluble in water and methanol.

Category: Synthetic codeine analogue central analgesic

λ_{max}: 270 nm

Drugs Used:

Table 1: List of Drugs Used

S. No	Drugs	Manufacturer
1.	Tramadol HCl	Vivan life sciences, Mumbai
2.	Tramadol HCl Commercial Tablets (Urogendol XL 200mg tablets)	Win Medicare Limited

Reagents Used:

Table: 2. List of Reagents used

S. No.	Chemicals	Manufacturer Name	Grade
1	Water	Merck	HPLC
2	Methanol	Merck	HPLC
3	Acetonitrile	Merck	HPLC
4	Ammonium acetate	Merck	G.R
5	Glacial acetic acid	Merck	G.R

Equipment and Apparatus Used:

Table 3: Equipment and Apparatus Used

S. No.	Instrument Name	Model Number	Software	Manufactures Name
1	HPLC	Alliance UV-Visible detector-2487	Empower	Waters
2	U.V Double beam spectrophotometer	SL 210	-	ELICO
3	Digital weighing balance (Sensitivity 5 mg)	BL-200H	-	SHIMADZU
4	PH-meter	LI-120	-	ELICO
5	Sonicator	3305013	-	SISCO

Preparation of mobile phase:

A combination of mobile phase containing 5mM ammonium acetate buffer (pH 4.0 ± 0.3): acetonitrile (15: 85 v/v) was mixed and degassed in ultrasonic water for 5 minutes finally filtered through 0.45µ membrane filter. This prepared solution was used as mobile phase

Diluent:

HPLC grade water and acetonitrile in the ratio of 50:50 (v/v) was used as diluent.

Preparation of standard solution: (0.4 mg/ml)

Accurately weighed 25 mg of Tramadol HCl working standard into a 25 mL volumetric flask, added 10 mL of diluent, mixed to dissolve and made up the volume with diluent. Further dilution was made by diluting 10 mL of the stock solution to 25 mL.

Preparation of sample solution :(0.4 mg/ml)

20 tablets were crushed to powder, weighed and transferred the tablet powder equivalent to 25 mg of Tramadol into 25 mL volumetric flask added 50 mL of diluent, sonicated for 10 minutes and diluted to volume with diluent. Filtered the solution through 0.45 µ nylon filter. Diluted 10 mL of the sample solution to 50 mL with diluent, prior to injection on chromatographic system.

A small portion of the extract (say 10 ml) was withdrawn and filtered to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent.

Wavelength selection:

About 0.25 mg/mL of Tramadol solution was accurately prepared by dissolving standard in water. The Tramadol solution was scanned in the 200-400 nm UV region. The wavelength maximum (λmax) was observed at 270 nm and this wavelength was adopted for absorbance measurement.

Optimized chromatographic conditions:

Column: Zorbax C18 column, 150 X 4.6 mm, 5µ
Column temperature: Ambient
Wave length: 270nm
Mobile phase ratio: 5mM ammonium acetate buffer: acetonitrile (15: 85 v/v)
Flow rate: 0.8 min/ml
Injection volume: 20µl
Run time: 2.5 minutes

Validation of developed RP-HPLC method:
As per the International conference on harmonization (ICH) guidelines the method validation parameters such as linearity, precision, accuracy, system suitability, limit of detection and limit of quantitation were optimized.

Assay
Sample and standard solutions were injected into the chromatographic system and measured the area for Tramadol and calculated the % assay by using the below formula:

Calculation:

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where :
Avg.wt = average weight of tablets
P = percentage purity of working standard
LC = label claim of Tramadol mg

RESULTS AND DISCUSSION:

Optimized method:
It was performed on Zorbax C18 column, 150 X 4.6 mm, 5µ with a mobile phase composition of 5 mM ammonium acetate buffer: acetonitrile (15: 85 v/v) at a flow rate of 0.8 min/ml. 20 µl of sample was injected and the run time was 2.5 minutes.

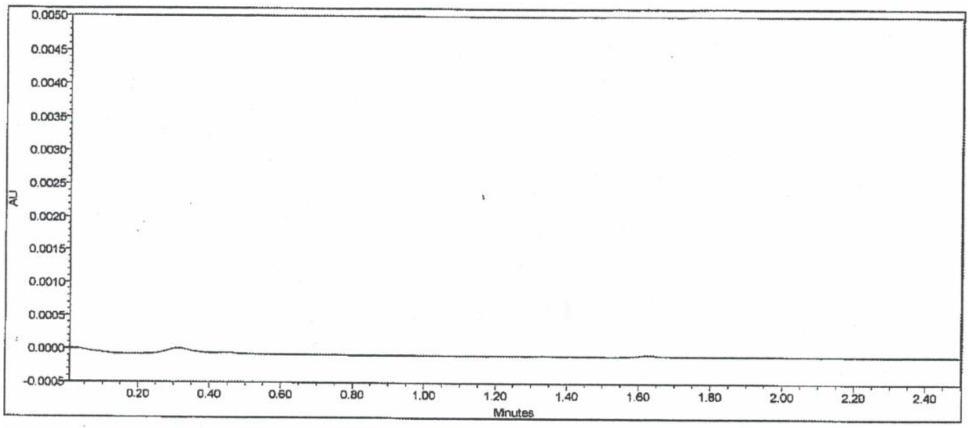

Fig. 2: Chromatogram showing blank preparation (mobile phase)

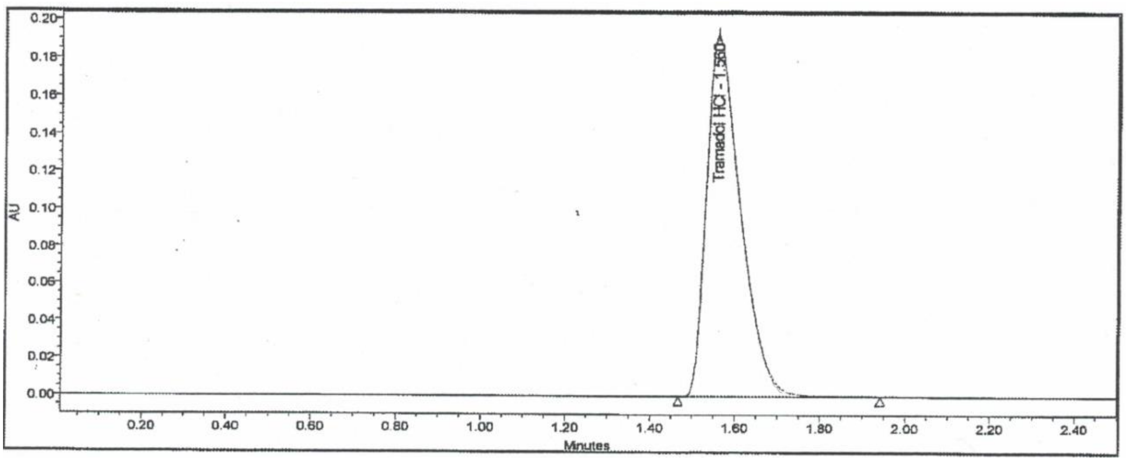

Fig. 3: Chromatogram of Tramadol HCl standard peak

Linearity:

0.2, 0.3, 0.4, 0.5, 0.6 mg/ml was injected into the chromatographic system and peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and Y-axis peak area) and the correlation coefficient was calculated.

Acceptance criteria:

Correlation coefficient should be not less than 0.999.

Table 4: Showing the results for the Linearity

Conc.(mg/ml)	RT	Area
0.2003	1.528	1102147
0.3005	1.522	1650299
0.4006	1.530	2204968
0.5008	1.526	2753639
0.6009	1.533	3302058
Co efficient of correlation(R^2)		0.9999

Precision:

The standard solution (0.4 mg/ml) was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria:

The % RSD for the area of five standard injections results should not be more than 2.

Table 5: Showing the results for Precision

S. No	Conc. (0.4 mg/ml)	RT	Area
1	100	1.523	2275087
2	100	1.524	2276216
3	100	1.524	2274801
4	100	1.524	2275014
5	100	1.523	2275732
Mean			2275370
SD			586.80
% RSD			0.0

Accuracy:

The standard solution of concentration 0.2, 0.4 and 0.6 mg/ml were injected into chromatographic system. Calculated % recovery and mean % recovery of Tramadol.

Acceptance criteria:

The % recovery for each level should be between 98.0 to 102.0%.

Table 6: Showing Accuracy results for Tramadol HCl

S. No	Conc(mg/ml)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean% recovery
1	0.2003	1103356	2	2.01	100.5%	
2	0.4006	2276356	4	4.02	100.5%	100.7%
3	0.6009	3302058	6	6.04	100.7%	

System suitability:

The standard I solution was injected one time and standard II solution was injected 5 times.

Table 7: Showing System Suitability results for Tramadol HCl

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	8352	1.1
2	1	8024	1.2
3	1.2	8765	1.2

Limit of detection (LOD)

From the above preparation 1ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluents.

Table.No.8. Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(mg/ml)
Tramadol HCl	671825.91	663365962	0.07

Limit of quantitation (LOQ)

From the above preparation 0.5ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluent.

Table.No.9. Showing results for Limit of Quantitation

Drug Name	Standard Deviation(σ)	Slope(s)	LOQ(mg/ml)
Tramadol HCl	671825.91	63365962	0.2003

Assay:

The developed and validated method was applied to the determination of Tramadol HCl in marketed tablets containing 4 mg of drug per tablet. Three injections of sample were injected into chromatographic system. Assay % was calculated by using the formula mentioned above and it was found to be 99%.

Table 10: Showing the results of assay

S. No	Name	RT	Area
1	Tramadol HCl	1.526	695226
2	Tramadol HCl	1.522	694341
3	Tramadol HCl	5.522	694434

CONCLUSION:

A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of Tramadol HCl in pure form and in tablets. The analytical conditions and solvent system developed provided a good separation for Tramadol HCl within a short analysis time. The method was validated and demonstrated a wide linear dynamic range, a good precision and accuracy. Thus, the method can be proposed for routine analysis in laboratories and for quality control.

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