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Research Article

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Method development and validation of visible spectroscopic method for the estimation of tramadol hydrochloride in pure and bulk dosage form

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ABSTRACT

The present research work discusses the development of visible spectroscopic method for the estimation of Tramadol Hydrochloride. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) was found to be 749nm. The validation was performed as per ICH guidelines for linearity, accuracy, precision, LOD and LOQ. The method shows high sensitivity with linearity in the range of 10-50µg/ml and shows a linear relationship between the absorbance and concentration with coefficient of correlation 0.998. The regression of curve was Y = 0.014x+0.016. The precision of method was found to be good. The percentage recovery was found to be 99.15±0.0950.The optimized method showed good reproducibility and recovery with RSD <2%. The proposed method will be suitable for analysis of Tramadol Hydrochloride in bulk as well as pharmaceutical formulations in quality control purpose. It is thus concluded that the proposed method is new, simple, cost effective, safe, accurate, precise and environmental friendly.

Keywords: Tramadol Hydrochloride, Visible spectroscopic method, Sensitive, Validation, ICH guidelines.

INTRODUCTION

Tramadol Hydrochloride is a centrally acting analgesic, used for treating moderate to severe pain. Tramadol Hydrochloride possesses agonist actions at the µ-opioid receptor and effects reuptake at the noradrenergic andserotonergic systems. Tramadol is a compound with µ-agonist activity. Chemically it is [2-(dimethylaminomethyl)-1-(3-methoxyphenyl) cyclohexanol]. It is used to treat moderate to moderately severe pain and most types of neuralgia, including trigeminal neuralgia. Tramadol is available in the form of oral drops, tablets, capsules and injections [1]. There are various methods available for estimation of Tramadol Hydrochloride like UV spectrophotometric[2,3], spectrofluorimetry[4],HPLC[5] , gas chromatography[6], GC-MS and LCMS[7], capillary electrophoresis[8], HPTLC[9],HPTLC-densitometry, [10,11] etc. However some of these methods are costlier and time consuming. To overcome these difficulties spectrophotometric analysis serves to be the quickest, promising and reliable method for routine analytical needs. The aim of the present study is to develop a new simple, rapid,reliable and precise visible spectrophotometric method for analysis of Tramadol Hydrochloride from tablet formulation; method is based on measurement of UV-visible absorbance of Tramadol Hydrochloride.

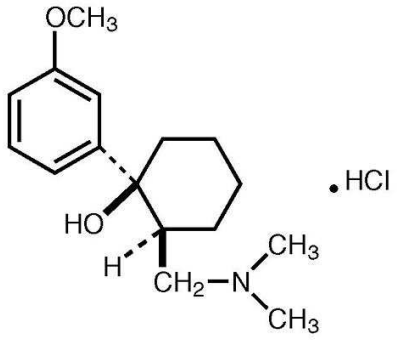

Fig. 1: Structure of Tramadol Hydrochloride

EXPERIMENTAL SECTION

Equipment and reagents

A LabIndia model 3000+ double beam UV-Visible Spectrophotometer with two matched cuvette cells of one cm light path were used for the measurement of absorbance. The Tramadol Hydrochloride bulk drug was kindly gifted by Hetero Labs Ltd., Hyderabad. The pharmaceutical dosage form was procured from market.

Preparation of standard stock solution

Accurately weighed 100 mg of Tramadol Hydrochloride was transferred into 100ml volumetric flask volume was made up to 100ml with water to get a concentration of 1000µg/ml.

Determination of λ_{max}:

From the stock solutions, 1ml of Tramadol Hydrochloride was transferred to 10 ml volumetric flask and the volume was adjusted to the mark with distilled water to obtain strength of 100µg/ml. From this solution 1ml of solution was transferred to 10ml volumetric flask. To this 1ml of 20% sodium carbonate solution and 0.5ml of Folin-Ciocalteu's reagent is added and the final volume was made up with distilled water. The solution was scanned in the UV-Visible range 200-800 nm**. (Fig. 2).**

Construction of calibration curve

Calibration curve was plotted against concentration and absorbance, regression equation was computed. The results tabulated in the table 1.

Preparation of Sample solution

Twenty capsules were weighed, average weight determined. Powder was removed from the shells. An accurately weighed quantity of powder equivalent to 100mg of Tramadol Hydrochloride was transferred into 100ml volumetric flask; volume was adjusted to mark with water. One ml was transferred to 10 ml volumetric flask; volume was adjusted to the mark. From this 1ml was taken into a 10ml volumetric flask. 1ml of 20%sodium carbonate solution and 0.5ml of Folin-Ciocalteu's reagent were added and the final volume was made up with the blank. The absorbance of this solution was recorded at 749nm.

METHOD VALIDATION

The proposed method was validated as per the ICH Q2 (R1) guidelines for linearity, accuracy, precision, LOD and LOQ [12].

Accuracy

Accuracy was carried out at 80 %, 100 % and 120 % of target concentration. From the amount found, percentage recovery was calculated.

Precision

Precision of the method was studied by carrying out intraday, interday analysis and expressed as percentage Relative Standard Deviation. For this purpose 10 (LQC), 30 (MQC) and 50µg/ml (HQC) solutions were prepared and the

absorbance's of the solutions were measured for six times within the same day and in different days at 749nm against blank.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ of the drug were calculated using the following equations according to International Conference on Harmonization (ICH) guidelines

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where σ = the standard deviation of the response and

S = the slope of the regression equation.

RESULTS AND DISCUSSION

The proposed method for determination of Tramadol Hydrochloride in marketed formulation (capsule) showed Sandal's sensitivity of 0.0401µg/cm^2/0.001 absorbance units. Linear regression of absorbance on concentration gave the equation y = 0.014x + 0.016 with a regression co-efficient (r^2) of 0.998 and the linearity range was 10- 50µg/ml. The higher percentage recovery value (95-105%) indicates that there is no interference of the excipients present in the formulation. Thus the method is useful for the determination of Tramadol Hydrochloride in bulk and pharmaceutical formulations.

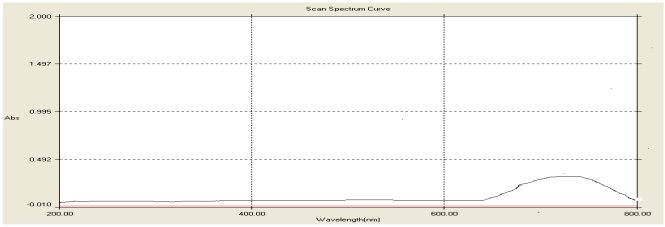

Fig.2: Spectrum of Tramadol Hydrochloride

Table 1: Calibration of proposed method

S.NO.	Conc. (mcg / ml)	Absorbance at 749nm
1	10	0.172
2	20	0.315
3	30	0.457
4	40	0.601
5	50	0.731

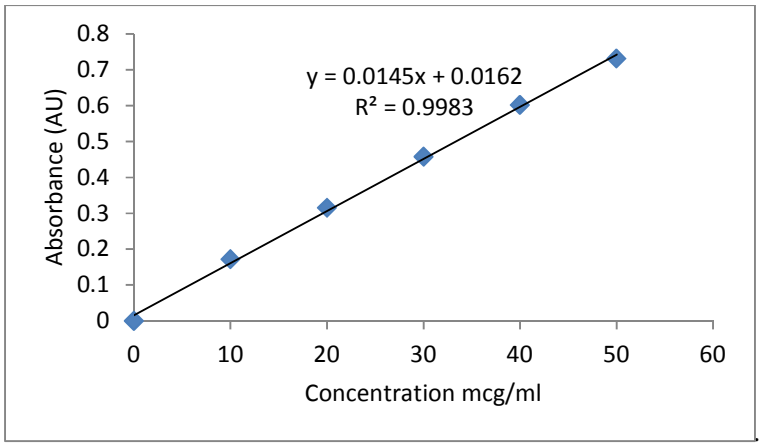

Fig.3.Calibration Curve of Tramadol Hydrochloride at 749nm

Table 2: Optimum conditions, Optical characteristics and Statistical data of the Regression equation in visible spectroscopic method

Parameter	colorimetric method
λ_{max} (nm)	749
Beer's law limits (mcg/ml)	10-50
Sandell's sensitivity (mcg/cm^2-0.001 absorbance units)	0.0401mcg/ml
Regression equation (Y*)	y =0.014x+0.016
Slope (b)	0.014
Intercept (a)	0.016
Correlation coefficient(r^2)	0.998
% RSD**	< 2%
Limit of detection (mcg/ml)	0.589
Limit of quantitation (mcg/ml)	1.787

*Note: *Y=bX + a where X is the concentration of Tramadol Hydrochloridein mcg/ml and Y is the absorbance at the respective $\lambda_{max.}$*
***Average of six determinations.*

Table 3:Assay of Tramadol Hydrochlorideformulation

S.No	Formulation	Label claim (mg/tab)	Amount found (mg) (n=3) Mean ± SD	Assay	%RSD
1	**Tramazac**	50mg	50.96±0.0802	101.92	0.157

Table 4: Determination of Accuracy results for Tramadol Hydrochloride

Brand name	Amount of sample (mcg/ml)	% of Spiked sample	Amount of drug added (mcg/ml)	Amount Recovered	% Recovery ± SD
Tramazac	1.5	80	1.2	28.18	104.01±0.0624
Tramazac	1.5	100	1.5	29.45	98.16± 0.0832
Tramazac	1.5	120	1.8	31.45	95.3±0.0781

Table 5: Determination of Precision results for Tramadol Hydrochloride

Conc. mcg / ml	Inter-day Absorbance Mean ± SD**	% RSD	Intra-day Absorbance Mean ± SD**	% RSD
LQC (10mcg/ml)	0.208± 0.00057	0.274	0.207 ±0.00057	0.275
MQC (30mcg/ml)	0.489± 0.00057	0.116	0.489±0.00057	0.116
HQC (50mcg/ml)	0.727±0.004	0.550	0.725±0.001	0.537

*Note: **Average of six determinations.*

CONCLUSION

A simple, sensitive, accurate and precise visible spectroscopic method has been developed for quantitative determination of Tramadol Hydrochloride in bulk and Pharmaceutical dosage form (capsule). The solution was scanned between 200 to 800 nm and 749 nm was selected as maximum wavelength for absorption. Beer's law was obeyed in the concentration range of 10–50µg/ml. % Recovery was calculated, was found to be 95.3 – 104.01 and the method was successfully applied to the pharmaceutical dosage form containing the Tramadol Hydrochloride drug without any interference by the excipients. The method was fast and economical and it was also selective and sensitive for the desirable range. Results of the analysis were validated as per ICH guidelines and by recovery studies.

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REFERENCES

[1] Anonymous. The Martindale Extra Pharmacopoeia.**1985**; EP 31: 1742-1744.
[2] HE Abdellatef. *Journal of Pharmaceutical and Biomedical Analysis*.,**2002**, 29(5), 835-842.

[3] M Puranik, AHirudkar, SJ Wadher,PG Yeole.. *Indian J. Phar.Sci*.,**2006**,68(6), 737-739.
[4] HE Abdellatef,MM El-Henawee, HM El- Sayedand MM Ayad. *Spectrochimica Acta A Molecular and Biomolecular Spectroscopy*.,**2006**, 65(5),1087-1092.
[5] S Negro, ASalama, Y Sanchez, ML Azuara,E Barcia. *Journal of Clinical Pharmacology and Therapeutics.*, **2007**,32(5), 441-444.
[6] Q Tao, DJ Stone, MR Borenstein, V Jean-Bart,EE Codd, TP Coogan, D Desai-Krieger, S Liao,RB Raffa. *Journal ofChromatography B: Biomedical Science Applications*.,**2001**, 763(1-2), 165-171.
[7] C Moore, L Marinetti, C Coulter,K *Forensic Science International*.,**2008**, 176(1), 47-50.
[8] J Li, HJu. *Electrophoresis*. **2006**, 27(17), 3467-3474.
[9] J Krzek, M Starek. *BiomedicalChromatography*.,**2004**, 18(8),589-599.
[10] B Ahrens, D Blankenhorn, B Spangenberg. *Journal ofChromatography B Analytical Technology inBiomedical and Life Sciences.*,**2002**, 772(1), 11-18.
[11] KVenkateshwarlu, YN Reddy, K Srisailam,V Rajkumar,MG Pai. *Current Trends in Biotechnology and Pharmacy*.,**2008**, 2 (3),421 -425.
[12] International Conference on Hormonization,Guidance for Industry in; Q2B Validation ofAnalytical Procedures: Methodology. **1996**: 2.