ANALYTICAL METHOD DEVELOPMENT AND VALIDATION STUDIES OF TRAMADOL HYDROCHLORIDE

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ABSTRACT

A simple, sensitive and reproducible UV Spectroscopic method was developed for the quantitative determination of Tramadol Hydrochloride. The λ_{max} of the tramadol hydrochloride was observed and recorded to be 271.00nm in distilled water as well as in the phosphate buffer pH 6.8 and it complies with the requirement of the standard texts. Correlation coefficientR^2in distilled water was 0.998 and of buffer solution was found as 0.997.The method was validated as per ICH Q2 (R1) guidelines and confirmed a good performance complies with respect to linearity, accuracy, and precision, range limit of detection and limit of quantification. So the proposed method can be used in routine quality control laboratories.

Key Words: *Tramadol hydrochloride, UV spectroscopic, Validation, ICH Q2 (R1) guidelines.*

INTRODUCTION

Tramadol is a centrally acting synthetic opioid analgesic. It acts as a weak agonist on all types of opioid receptors with some selectivity for the M receptors.[1] from animal tests; at least two complementary mechanisms appear applicable: binding of parent and M1 metabolite to μ-opioid receptors and weak inhibition of reuptake of nor epinephrine and serotonin. Tramadol has been shown to inhibit reuptake of nor epinephrine and serotonin in vitro, as have some other opioid analgesics. These mechanisms may contribute independently to the overall analgesic profile of tramadol. Tramadol is extensively metabolized. The production of the only known active metabolite, M1 (mono-o-desmethyl tramadol) is dependent on the CYP2D6 isoenzyme of the cytochrome P-450 enzyme system and hepatic impairment results in decreased metabolism of both the parent compound and the active metabolite. Patients who metabolize drugs poorly via CYP2D6 may obtain reduced benefit from tramadol due to reduced formation of M.[2]

Tramadol is extensively metabolized after oral administration. Approximately 30% of the dose is excreted in the urine as unchanged drug, whereas 60% of the dose is excreted as metabolites. The major metabolic pathways appear to be N- and O-demethylation and glucuronidation or sulfation in the liver. Tramadol should be used with caution in patients with increased intracranial pressure or head injury. Tramadol is used to treat moderate to moderately severe pain and most types of neuralgia, including trigeminal neuralgia. It has been suggested that tramadol could be effective for alleviating symptoms of depression, anxiety, and phobias because of its action on the noradrenergic and serotonergic systems. Tramadol is used to treat post-operative, injury-related, and chronic (e.g., cancer related) pain in dogs. Adverse drug reactions are like dizziness/vertigo, nausea, vomiting and headache.[3,4]

Concomitant administration of tramadol with caramazepine causes a significant increase in tramadol metabolism.[5] Tramadol is metabolized to M1 by the CYP2D6 P-450 isoenzyme. Quinidine is a selective inhibitor of that isoenzyme, so concomitant administration of quinidine and tramadol results in increased concentration of tramadol and reduced concentration of M1. Concomitant administration with inhibitors of CYP2D6 such as fluoxentine, paroxentine, and amitryptiline could result in some inhibition of the metabolism of tramadol. Interactions with MAO inhibitors, due to interference with detoxification mechanisms enhance seizure risk.[6, 7]

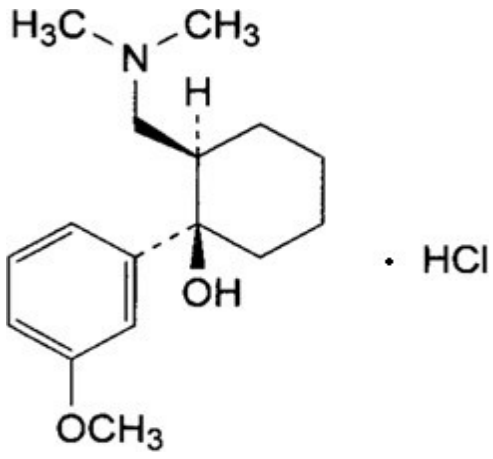

Fig. 1: Structure of Tramadol Hydrochloride

MATERIALS AND METHODS

Tramadol hydrochloride, water, phosphate buffer andU.V.-1800 series spectrophotometer, 1cm quartz cell of Shimadzu was provided by B.N. College of pharmacy, Udaipur.

Preparation of stock solution for tramadol hydrochloride in distilled water: Standard stock solution: Accurately weighed 100 mg tramadol hydrochloride was transferred in 100 ml volumetric flask. It was dissolved in distilled water and volume was made up to 100 ml.

A series of tramadol hydrochloride solution in the range of 10to 100 µg/ml were prepared from standard stock solution. The absorbance of all solutions was measured against blank at λ_{max} by UV spectrophotometer.

Preparation of stock solution for tramadol hydrochloride in phosphate buffer of pH 6.8: Standard stock solution: Preparation of pH 6.8 (0.1 M) phosphate buffer: Accurately weighed 27.2 mg of potassium dihydrogen phosphate was dissolved in 250 ml distilled water, mixed and added 112 ml of 0.2 M sodium hydroxide; volume was adjusted with water to 1 L and mixed well.

Accurately weighed 100 mg of tramadol hydrochloride was transferred in 100 ml volumetric flask. It was dissolved in pH 6.8 buffer solution and volume was made up to 100 ml.

A series of the tramadol hydrochloride solution ranging from 10 to100 µg/ml were prepared from standard stock solution. Absorbance of all solutions was measured by UV spectrophotometer at 271nm.

Validation Parameters:

Accuracy: System suitability solution was prepared as given above. Blank, system suitability solution was injected as per sequence and the acceptance criteria i.e. %RSD for system suitability was checked. Accuracy (recovery) was carried out at 80%, 100% and 120% of target limit.

Precision: Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples. According to the ICH, precision should be performed at three different levels: system precision, repeatability and intermediate precision. Individual and cumulative (overall) % of individual impurity found within acceptance limit (%RSD should NMT 10) hence the method is rugged.

Linearity and Range: For the calibration standards, dilutions 10 to 100µg/ml were prepared from stock solutions. The dilutions were prepared and scan by UV Spectroscopy.

Limit 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 X σ/S,
Where, σ = the standard deviation of the response
S = slope of the calibration curve.

Limit 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 X σ/S
Where, σ = the standard deviation of the response
S = slope of the calibration curve

RESULT AND DISCUSSION

Ultraviolet Absorption Maxima scan (λ_{max}): The UV absorption spectra were observed as presented in Figure 2-3. The λ_{max} of the tramadol hydrochloride was observed and recorded to be 271.00nm in distilled water as well as in the phosphate buffer pH 6.8andit complies with the requirement of the standard texts.

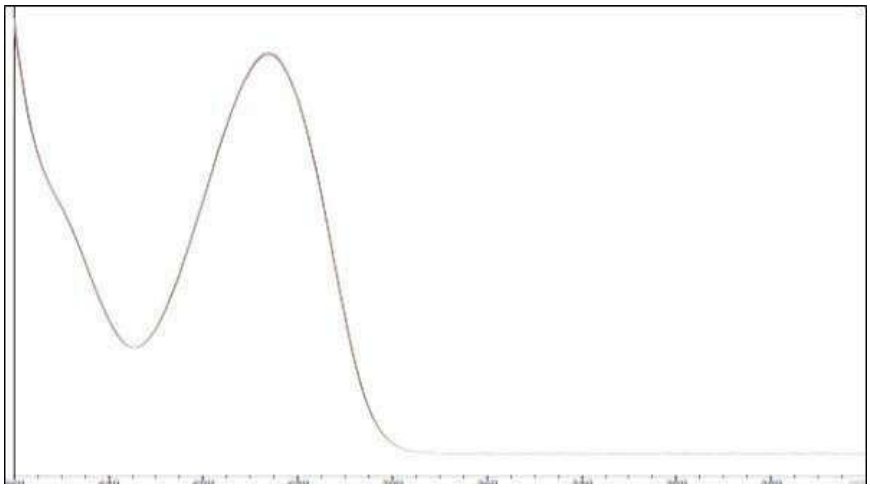

Fig. 2: UVabsorption maxima of tramadol hydrochloride in distilled water

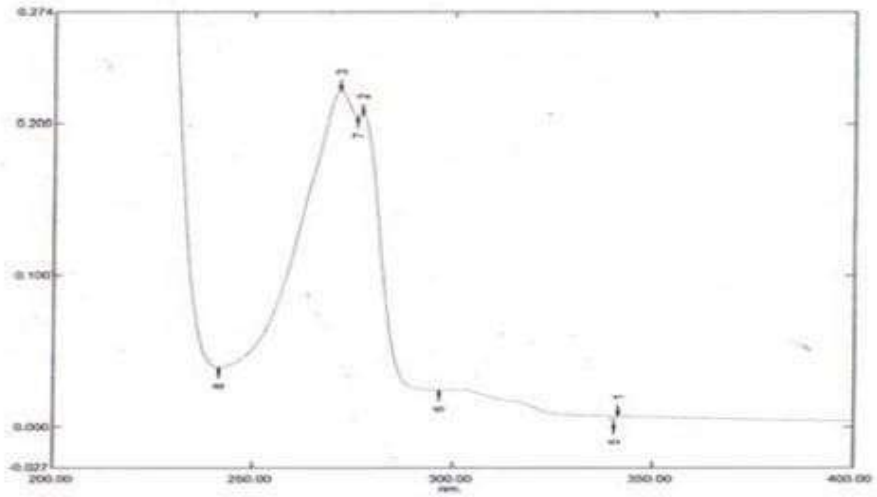

Fig. 3: UVabsorption maxima of tramadol hydrochloride in buffer pH 6.8

Validation Parameters

Accuracy: Accuracy for individual and mean at each level between 80 % to 120 % as the data shown in Table 1.

Table 1: Percent recoveries by UV methods of analysis

Concentration	Distilled water		% Recovery	tramadol hydrochloride in buffer pH 6.8		
	Mean	% RSD		Mean	% RSD	% Recovery
80%	0.0202	0.28	97.016	0.2351	0.42	99.23
120%	0.4056	0.05	99.71	0.4313	0.76	98.32
120%	0.6044	0.32	99.81	0.7023	0.73	99.26

Precision: Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples. According to the ICH, precision should be performed at three different levels: system precision, repeatability and intermediate precision. Individual and cumulative (overall) % of individual impurity found within acceptance limit (%RSD should NMT 10) hence the method is rugged data shown in table 2.

Table 2: Study of Precision Parameter by UV in Distilled water

Method	Parameters	System Precision	Method Precision	Intermediate Precision	
				Inter-day	Intraday
UV	Mean* ± SD	0.3985 0.031	0.4251 0.004	0.4042 0.003	0.4067 0.003
	% RSD	0.8	1.0	0.88	0.73

**n=6*

Table 3: Study of Precision Parameter by in UV buffer pH 6.8

Method	Parameters	System Precision	Method Precision	Intermediate Precision	
				Inter-day	Intraday
UV	Mean* ± SD	0.3571 0.028	0.4987 0.005	0.4222 0.006	0.4231 0.004
	% RSD	0.9	1.3	0.81	0.70

**n=6*

Calibration curve for tramadol hydrochloride distilled water

The standard calibration curves of tramadol hydrochloride were constructed by plotting absorbance vs. concentration in distilled water and phosphate buffer of pH 6.8 media. The results were averaged and analyzed by simple linear regression model $y = mx + c$. The linearity range was found 10-100 µg/ml as illustrated in table 4 and figure 4-5.

Table 4: Calibration curve data for tramadol hydrochloride in distilled water

Concentration (µg/ml)	Absorbance in distilled water	Absorbance phosphate buffer of pH 6.8
10	0.068	0.062
20	0.124	0.112
30	0.179	0.168
40	0.232	0.224
50	0.289	0.275
60	0.368	0.348
70	0.426	0.412
80	0.478	0.465
90	0.544	0.526
100	0.62	0.562
λ_{max}	271 nm	271 nm
Linear range	10-100(µg/ml)	10-100(µg/ml)
Regression equation	y = 0.0061x - 0.0027	y = 0.0058x - 0.0019
Correlation coefficient R^2	0.998	0.997

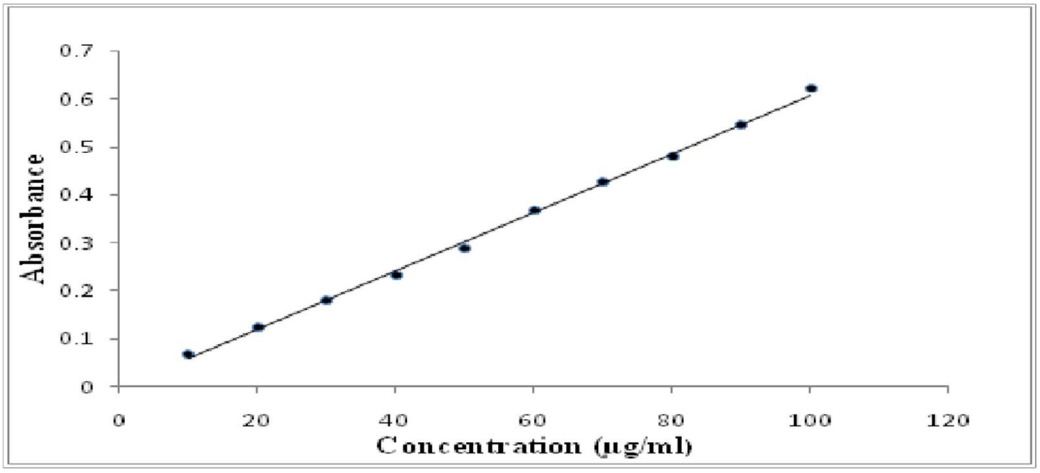

Figure 4: Calibration curve for tramadol hydrochloride in distilled water

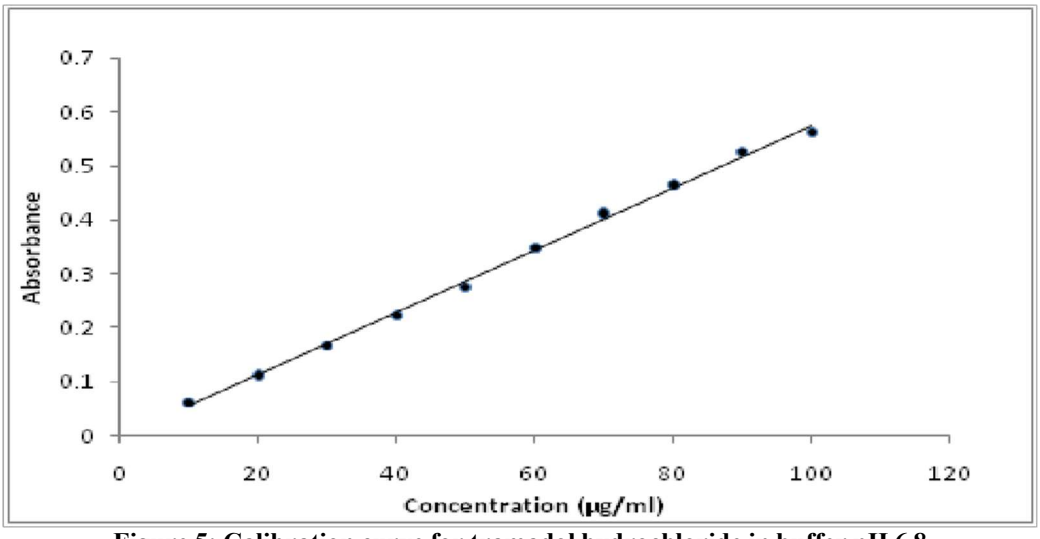

Figure 5: Calibration curve for tramadol hydrochloride in buffer pH 6.8

Limit of detection: The limit of detection (LOD) of the tramadol hydrochloride was calculated by using equations for tramadol hydrochloride in distilled water and tramadol hydrochloride in buffer pH 6.8. So the lowest level of concentration can be detected by method and was found as 0.22and 0.31 respectively.

Limit of quantification: The limit of quantization (LOQ) of the tramadol hydrochloride was calculated by using equations for tramadol hydrochloride in distilled water and tramadol hydrochloride in buffer pH 6.8.So the lowest level of concentration can be quantified by method and was found as 1.4and 086.

SUMMARY AND CONCLUSION

The UV spectroscopy Recovery studies were carried out by adding known quantities of standards at different levels (80 to120 %) to the pre-analyzed sample to study the linearity, accuracy, precision, LOQ, LOD. The recovery studies also reveals whether there is a positive or negative influence on the quantification parameters by the additives usually present in the dosage forms. 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, linearity, accuracy and precision range over the concentration range examined.

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REFERENCES

1. Ho WH and Lee HLV. Sustained Drug Delivery Fundamentals and Applications: Design and fabrication of oral controlled release drug delivery system. 2nd ed. Marcel Dekker Inc, New York, 1987, pp 373-420.
2. Kar RK, Mohapatra S and Barik BB. (2009) Design and characterization of controlled release matrix tablets of zidovudine. Asian J Pharm Cli Res. 2:54-6.
3. Amidon GL, Lennernas H, Shah VP and Crison JR. (1995) A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability, Pharm. Res.12: 413-420.
4. Loèbenberg R and Amidon GL. (2000) Modern bioavailability, bioequivalence and Biopharmaceutics classification system. New scientific approaches to international regulatory standards. European Journal of Pharmaceutics and Biopharmaceutics. 50: 3-12.
5. Reddy BK, and Karunakar A. (2011) Biopharmaceutics Classification System: a regulatory approach. Dissolution Technologies.2:31-37.
6. Patrick JS. Martin's Physical Pharmacy and Pharmaceutical Sciences. 3rd ed. Varghese Publishing House. Bombay: 1991: 512-519.
7. Mohammed AD, James LF, Michael HR, John EH and Rajabi-Siahboomi AR. (1999) Release of propranolol hydrochloride from matrix tablets containing sodium carboxy methylcellulose and Hydroxypropyl methylcellulose. Phar. Dev. Tech. 4:313-324.