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UV Spectrophotometric Method: A Quantitative Estimation of Tolperisone Hydrochloride in Bulk and Pharmaceutical Dosage Form

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Abstract: A rapid, specific and sensitive UV spectrophotometric method was developed for the determination of Tolperisone hydrochloride in bulk and tablet dosage form. The absorbance was measured at 260 nm using purified water as a solvent and the calibration curve was found to be linear in the concentration range of 3 – 18 µg/ml. The molar absorptivity and Sandell's sensitivity were obtained 1.33 X 10^4 liter/mol/cm and 0.019 µg/cm^2/0.001 absorbance units respectively. The detection limit and quantitation limit were found to be 0.032 and 0.096 µg/ml respectively. The % recovery was detected 99.09 – 101.26 %. The method was found to be precise as the value of % RSD was remained below 2 %. There was not found any interference of common excepients used in the tablet formulation. Hence the proposed UV spectrophotometric method seems to be suitable for the routine quality control analysis in analytical lab.
Keywords: Tolperisone Hydrochloride, Spectrophotometry, Method Validation, Bulk and Pharmaceutical formulation.

INTRODUCTION

Tolperisone Hydrochloride (TOLP), chemically 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one monohydrochloride, is a piperidine derivative[1,2] (Figure 1). It has a specific relaxant effect on disabling neuromuscular spasms and thereby significantly improves patients' mobility with any sedative side effects[3]. TOLP acts at the level of spinal cord by blocking sodium channels and calcium channels[4]. TOLP exerts its spinal reflex inhibitory action predominantly via a pre synaptic inhibition of the transmitter release from the primary afferent endings via a combined action on voltage-gated sodium and calcium channels[5]. It is only with Sanochemia's developments, now licensed to Avigen, that the potential of TOLP can be fully utilized[3]. TOLP has the unique property of mediating muscle relaxation without concomitant sedation and it does not cause incoordination, weakness and mental confusion or withdrawal phenomena, in contrast to other muscle relaxants[6].

Japanese Pharmacopoeia suggests a potentiometric titration for the determination of TOLP in bulk[7]. A number of methods such as colorimetry[8], HPLC[9,10], LCMS[11] were reported for the quantitative estimation of TOLP. Potentiometric titration is an accurate but highly tedious method and no other official methods have been reported. Hence it was decided to develop and validate a sensitive, accurate and rapid spectrophotometric method for determination of TOLP in bulk and pharmaceutical dosage form so as to fulfill the requirements of routine analysis in quality control department of pharmaceutical company.

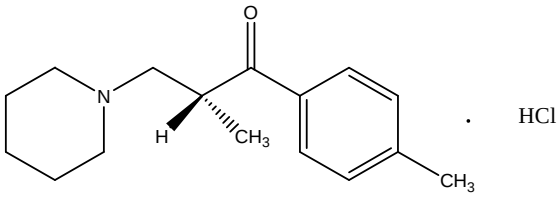

Figure 1: Chemical structure of TOLP

MATERIALS AND METHODS

Instruments

UV-Visible double beam spectrophotometers (UV-1700, Shimadzu Co., Japan and Lambda 19, Perkin Elmer, USA) with 1 cm matched quartz cells and Digital balance (Mettler Toledo, UK) were used.

Chemicals and Reagents

TOLP was procured as gratis sample from Themis Medicare Ltd., Vapi, Gujarat. The obtained TOLP was having 99.90% w/w assay value and was used without further purification. All solutions were prepared with purified water of I.P. grade[12]. The TOLP tablets were purchased from local market (TOLPIDOL®, Themis Medicare and TOLFREE®, Zydus Cadila).

Preparation of standard stock solution (100 µg/ml)

The standard stock solution was prepared by transferring 25 mg standard TOLP in to a 25 ml volumetric flask. 10 ml purified water was transferred to this volumetric flask and dissolved. The volume was made up to the mark with purified water to give a solution containing 1000 µg/ml TOLP. From this solution 1.0 ml was transfer to 10 ml volumetric flask and the volume was adjusted to the mark with the purified water to give a solution containing 100 µg/ml TOLP.

Calibration curve for TOLP (3 – 18 µg/ml)

Appropriate volumes i.e. 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 ml from stock solution were transferred to different volumetric flasks of 10 ml capacity. The volumes were adjusted to the mark with the purified water to obtain concentrations of 3, 6, 9, 12, 15 and 18 µg/ml. Absorbance was measured at selected wavelength and absorbance vs. concentration graph was plotted. The value of regression coefficient was calculated by least square method.

Determination of TOLP in pharmaceutical dosage form (Label Claim: 150 mg)

Twenty tablets were weighed and finely powered. Powder equivalent to 25 mg TOLP was accurately weighed and transferred to volumetric flask of 25 ml capacity. 10 ml purified water was transferred to this volumetric flask and dissolved. The volume was made up to the mark with purified water. The solution was filtered through 0.45µ filter paper. 0.1 ml of this solution was transferred to volumetric flask of 10 ml capacity and volume was made up to the mark. The absorbance of prepared solution was measured at detection wavelength for quantification of TOLP.

Method Validation

Developed method was validated as per ICH guidelines[13]. Accuracy was determined by standard addition method i.e. addition of 80%, 100% and 120% of target concentration (10 µg/ml). Precision of developed method was evaluated by repeatability, intra day and inter day study and percentage relative standard deviation (% RSD) was calculated. Detection limit (LOD) and quantitation limit (LOQ) were measured as per ICH guidelines. The reproducibility was confirmed by measuring absorbance at different laboratory using another spectrophotometer by another analyst and the values obtained were evaluated using t- test. The specificity of the method was checked by monitoring a standard solution of TOLP in presence of excipients of tablets at the same concentration levels as used in tablets using the method described in the procedure for calibration curve.

RESULTS AND DISCUSSION

The method was developed in purified water as the solubility of TOLP in the purified water and also there was no shift in the absorbance maxima of TOLP in purified water. From overlain spectra of TOLP (Figure 2) it was clear that TOLP exhibited significant absorbance at 260 and hence 260 nm was considered as detection wavelength. Statistical data at 260 for TOLP are shown in Table 1. Calibration curve for linear range of 3 – 18 µg/ml of TOLP was obtained as straight line with 0.999 regression coefficient as shown in Figure 3. The % recovery of TOLP was found in range of 99.09 – 101.26 % which falls within limit (98% - 102%) as mentioned in Table 2. The % RSD for repeatability study was found to be 0.387 – 1.522 and 0.216 for bulk and tablet dosage form respectively. The data for repeatability study are tabulated in Table 3 and 4. The acceptable limit for repeatability study is below

2 % RSD value. The observations of intraday and interday precision study were obtained well within limit as shown in Table 5. The t-test was performed on the two data set obtained from two different instruments and compared with tabulated value. There was no significant difference was observed as mentioned in Table 6. The values of LOD and LOQ obtained are 0.032 µg/ml and 0.096 µg/ml respectively (Table 7). The LOD and LOQ values indicate that method can detect and also quantify a minute quantity of TOLP in sample. It was observed that excipients were not interfered in the analysis of TOLP.

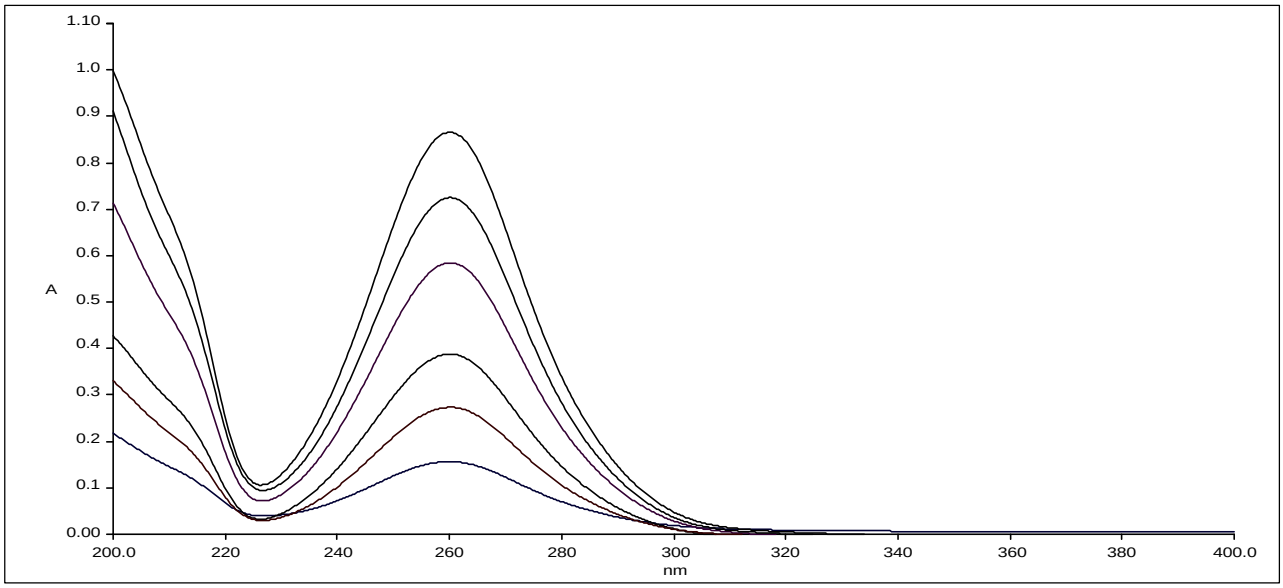

Figure 2: Overlain Spectrum of TOLP in Purified Water

Table 1: Statistical data of TOLP at 260 nm

Parameter	**TOLP (at 260 nm)**
Linear Range (µg/ml)	3 - 18
Slope of the curve	0.047
Intercept of the curve	0.003
Standard deviation of slope	0.0004
Limit of Detection (µg/ml)	0.032
Limit of Quantitation (µg/ml)	0.096
Regression equation	Y = 0.047x + 0.003
Correlation coefficient (r^2)	0.999

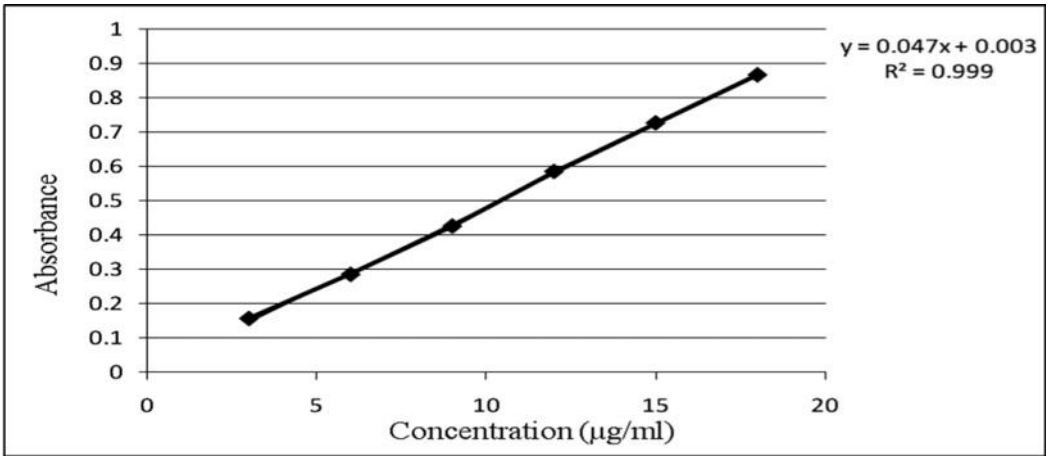

Figure 3: Calibration Curve of TOLP in purified water at 260 nm

Table 2 Determination of Accuracy for TOLP by Spectrophotometry

Amount of drug in sample (mg)	Amount of standard drug added (mg)	Amount measured (mg)	Amount recovered (mg)	% Recovery	Average of % recovery
10	0	-	-	-	-
10	8	17.95	7.95	99.40	99.54
10	8	17.96	7.96	99.51	
10	8	17.97	7.97	99.70	
10	10	20.12	10.11	101.17	100.59
10	10	20.04	10.04	100.45	
10	10	20.02	10.01	100.16	
10	12	21.78	11.96	99.70	100.02
10	12	21.89	11.89	99.09	
10	12	21.85	12.15	101.26	

Table 3 Repeatability study of TOLP in Bulk form

Concentration	6 (µg/ml)	9 (µg/ml)	12 (µg/ml)
Absorbance	0.280	0.428	0.586
	0.286	0.423	0.582
	0.284	0.426	0.583
Mean.	0.283	0.426	0.584
Std. Dev.	0.004	0.003	0.002
% RSD	1.522	0.638	0.387

Table 4 Repeatability study of TOLP tablet

Concentration	10 (µg/ml)
Absorbance	0.467
	0.467
	0.468
	0.467
	0.465
	0.465
Mean	0.467
Std. Dev.	0.001
% RSD	0.216

Table 5 Intra day and Inter day study of TOLP

Concentration (µg/ml)	Intra day (n =3)	% RSD	Inter day (n =3)	% RSD
6	0.282 ± 0.003	0.970	0.297 ± 0.005	1.683
9	0.423 ± 0.002	0.567	0.471 ± 0.008	1.699
12	0.585 ± 0.004	0.631	0.540 ± 0.007	1.297

Table 6 Reproducibility study of TOLP (9 µg/ml)

Instrument 1	Instrument 2	Result of t test*	Inference
0.429±0.013	0.457±0.019	0.022	No significant difference

Table 7 Determination of TOLP in marketed formulations

Formulation (Tablet)	Actual concentration (µg/ml)	Amount obtained (µg/ml)	% TOLP
Brand 1 (Tolpidol®)	10	9.89	98.91
Brand 2 (Tolfree®)	10	9.93	99.34

CONCLUSION

The discussed Spectrophotometric method provides an accurate, economical, ecofriendly and convenient method for the analysis of Tolperisone hydrochloride in bulk and in tablet dosage form. The proposed method may built a great value in quality control determinations of Tolperisone Hydrochloride because of its adequate accuracy, reliability for the determination of this drug in the pharmaceutical dosage forms without interference from commonly encountered excipients.

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