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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE ESTIMATION OF TOLPERISONE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

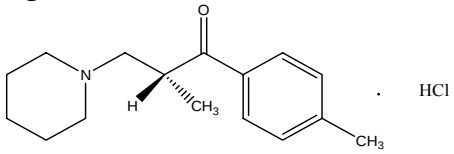
A rapid, specific and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for the determination of Tolperisone Hydrochloride (TOLP) in bulk and tablet dosage form. The method involved an isocratic elution of TOLP on C_{18} column (250 X 4.6 mm, 5 µm) using a mobile phase composition of acetonitrile: 20 mM ammonium acetate buffer containing 0.1% triethyl amine (55:45 v/v) (pH 4.0 adjusted by glacial acetic acid), at a 1.0 ml/min flow rate. The analyte was monitored at 260 nm wavelength. The retention time for tolperisone was found to be 2.50 min. Linearity was established in range of 12.5 - 100 µg/ml with correlation coefficient 0.999. The % recovery was obtained as 98.38 – 101.58 %. The detection limit and quantitation limit were found to be 0.172 and 0.521 respectively. This method can be successfully employed for quantitative analysis of TOLP in bulk and pharmaceutical dosage form as its accuracy, precision, specificity and reproducibility.

***Keywords*:** Tolperisone Hydrochloride, RP-HPLC, USP and ICH guidelines

1. Introduction

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 without any sedative side effects[3]. 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[4]. 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[5].

Figure 1: Chemical structure of TOLP



Japanese Pharmacopoeia suggests a potentiometric titration for the determination of TOLP in bulk[6]. A number of methods such as colorimetry[7], HPLC[8], LCMS[9] have been reported for the quantitative estimation of TOLP. Potentiometric titration is an accurate but highly tedious method and no other official methods have been reported. Also reported methods have some demerits i.e. long run time, somewhat higher use of hazardous solvents. Hence it was decided to develop and validate a sensitive, accurate and rapid RP-HPLC 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. In the present work the RP-HPLC method is emphasized for the estimation of TOLP.

2. EXPERIMENTAL

2.1 Materials and methods: TOLP was procured as a gratis sample from Themis Medicare Pvt. Ltd., Vapi, Gujarat. The chemical structure and purity of obtained TOLP were authenticated by Fourier Transform Infrared Spectroscopy (FT-IR) and melting point measurement. HPLC grade acetonitrile, ammonium acetate, triethyl amine (TEA), glacial acetic acid and water (Merck Ltd., Mumbai, India) were used in proposed research work. The TOLP tablets claimed to contain 150 mg were obtained from local market (Tolpidol®, Themis Medicare and Tolfree®, Zydus Cadila).

2.2 Instrumentation and chromatographic conditions: Perkin Elmer HPLC system has been employed to develop this method. System consists of a quaternary gradient pump (LC-200), manual loop injector (Rheodyne injector

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with fix 20 µl capacity), degasser unit and UV-visible detector (Series 200 LC). Chromatographic separations were carried out using the C_{18} column (250 X 4.6 mm, I.D. 5 µm) purchased from Chromatopak analytical instrumentations Ltd., India. Mobile phase consisted of acetonitrile: 20 mM ammonium acetate buffer containing 0.1% Triethyl amine (55:45 v/v) (pH 4.0 adjusted by glacial acetic acid). Mobile phase was filtered through 0.45 µ nylon filter and degassed in ultrasonic bath for 10 min before running the experiment. Flow rate was kept at 1 ml/min and the detection was carried out using 260 nm as detection wavelength.

2.3 Preparation of mobile phase: Accurately weighed 1.54 gm ammonium acetate was dissolved in 900 ml of water. 1.0 ml TEA was added in it and then pH was adjusted to 4.0 with glacial acetic acid. The volume was made up to 1000 ml with water. Prepared 20 mM ammonium acetate buffer containing 0.1% TEA (pH 4.0 adjusted by glacial acetic acid) was mixed with acetonitrile in the ratio of 55:45 v/v. Mixture was filtered through 0.45 µ nylon filter and sonicated for 10 minutes to degas the mixture.

2.4 Standard stock solution of TOLP: A 25 mg of standard TOLP was weighed and transferred to a 25 ml volumetric flask. 10 ml water was added in flask and sonicated for 10 min. The volume was made up to the mark with water to give a solution containing 1000 µg/ml TOLP.

2.5 Preparation of assay sample solution: Twenty tablets of TOLP were weighed and finely powdered. The powder equivalent to 25 mg TOLP was accurately weighed and transferred to volumetric flask of 25 ml capacity. 10 ml of water was transferred to volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with water. The above solution was filtered through 0.45µ nylon filter. 0.5 ml of aliquot was taken and transferred to volumetric flask of 10 ml capacity. The volume was made up to the mark with the diluent to give a solution of 50 µg/ml of TOLP.

2.6 Method validation: Developed method was validated as per USP[10] and ICH[11] guidelines.

2.6.1 System suitability: To assess system suitability of the method, the peak area, theoretical plates, tailing factor and retention time of six replicate injections of standard TOLP of concentration (50µg/ml) were used.

2.6.2 Linearity: The linearity was analyzed through the standard curves ranging from 12.5 - 100µg/ml [25% - 200% of target concentration of TOLP (50 µg/ml)] by diluting appropriate amounts of TOLP stock solution (1000µg/ml) with water and prepared in triplicate. Three calibration curves were prepared in the same day with the following concentrations (12.5, 25, 37.5, 50, 75 and 100µg/ml). The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

2.6.3 Specificity: 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.

2.6.4 Accuracy: Accuracy was determined by standard addition method i.e. addition of 80%, 100% and 120% of target concentration (50 µg/ml).

2.6.5 Precision: Precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) of both standard and sample solutions. Precision was determined in six replicates of both TOLP standard solution (50µg/ml) and sample solution (50µg/ml) on the same day (intra-day precision) and for 3 consecutive days (inter-day precision). The results were expressed as % RSD of the measurements.

2.6.6 Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ were determined using calibration curve method according to ICH Q_2B (R1) recommendations. The LOD (k = 3.3) and LOQ (k = 10) of the proposed method were calculated using the following equation:

$A = k\,\sigma / S$,

Where, A is LOD or LOQ, σ is the standard deviation of the response, and S is the slope of the calibration curve.

2.6.7 Reproducibility: 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.

2.6.8 Robustness: It was determined by studying the effect of flow rate at 0.9 and 1.1ml/min instead of 1.0 ml/min. The effect of mobile phase composition was assessed at (ACN : Buffer = 53:47v/v) and (ACN : Buffer = 57:43v/v) instead of (ACN : Buffer = 55:45v/v).

The %RSD of robustness testing under these conditions was calculated in all cases.

3. Results and Discussion

3.1 Method validation

3.1.1 System suitability: The results of the system suitability parameters are shown in Table 1, indicating the good performance of the system and better suitability for the analysis of drug.

Table 1: System suitability characteristics of proposed method.

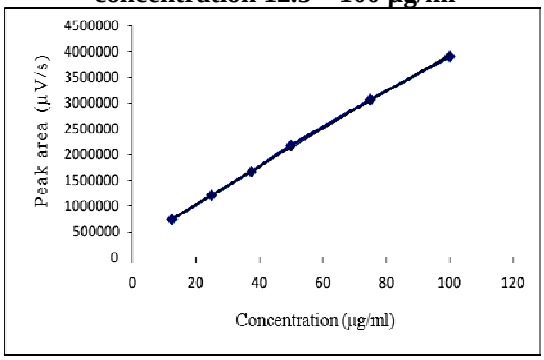
Parameter	Value Mean ±%RSD (n = 6)	Acceptable Limit
Peak area (µV/s)	2203484.635±0.011	-
Tailing factor	1.55±0.247	< 2
Theoretical plate	3336±1.094	> 2000
Retention time (min)	2.50±0.035	-

3.1.2 Linearity: The regression equation for TOLP was found $y = 37191x + 24225$ by plotting peak area (y) versus the concentration (x) studied from 12.5 - 100µg/ml and the correlation coefficient, 0.999 was highly significant. Results of three calibration curves are shown in Table 3 and linearity curve is presented in Figure 2.

Table 2: Area for linearity solutions at 260 nm for TOLP

Conc. (µg/ml)	Area Mean ± S.D. (n = 3)	% RSD
12.5	742302.002±113.841	0.017
25	1214744.376±133.514	0.012
37.5	1667319.627±1131.561	0.068
50	2180484.725±136.602	0.012
75	3072731.035±3816.042	0.124
100	3909298.306±6177.201	0.159

Figure 2: Linearity curve for TOLP concentration 12.5 – 100 µg/ml



3.1.3 Specificity: A typical HPLC chromatogram of TOLP standard preparation and TOLP test sample are shown in Figure 3(a) and 3(b). The HPLC chromatograms recorded for the mixture of the inactive ingredients revealed no peaks within retention time around 2.50 min and the peak purity was 99.99%. Figure 3(a) and 3(b) show that TOLP is clearly separated from the response of any interfering peak(s).

Figure 3(a): Typical chromatograms of TOLP standard preparation

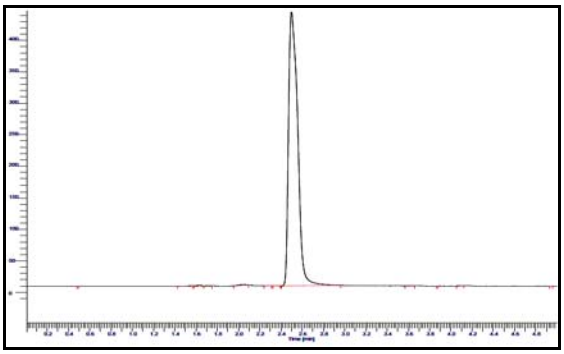
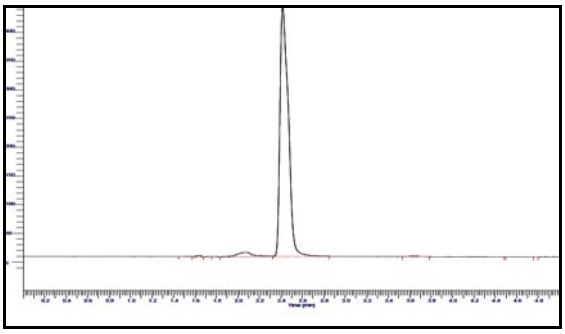


Figure 3(b): Typical chromatograms of TOLP sample preparation



3.1.4 Accuracy: The results of this study were expressed as % recoveries of the particular components in the samples. The % recovery of TOLP was obtained in range of 98.38 – 101.58 %. The results shown in Table 3, indicate good accuracy of the proposed RP-HPLC method.

Table 3: Accuracy study of TOLP by standard addition method

Amt of drug in sample (mg)	Amt of std drug added (mg)	Amt measured (mg)	Amt recovered (mg)	% Recovery	Average of % Recovery
50	0	49.19	-	-	-
50	40	90.09	40.09	100.23	99.80
50	40	89.93	39.93	99.82	
50	40	89.74	39.74	99.35	
50	50	99.95	49.95	99.90	100.54
50	50	100.62	50.62	101.24	
50	50	100.24	50.24	100.48	
50	60	110.61	60.61	101.01	100.32
50	60	109.03	59.03	98.38	
50	60	110.95	60.95	101.58	

3.1.5 Precision:; The values of %RSD for intraday and interday variation are given in Table 4. In both cases, %RSD values were found well within 2% limit, indicating that the current method is repeatable.

Figure 4(a): Typical chromatogram of TOLP at LOD concentration

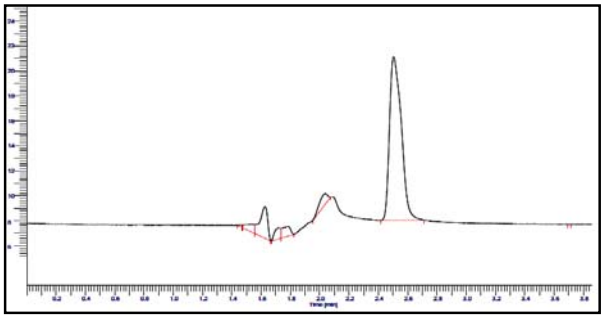
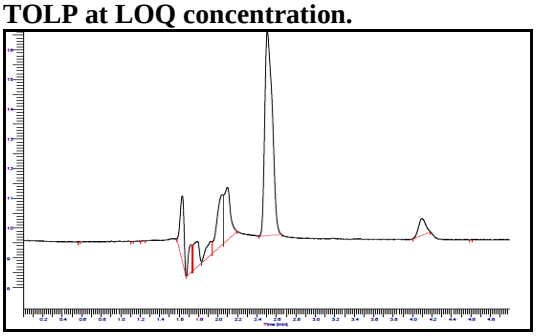


Table 4: Results of Repeatability (Intra-day) and Intermediate Precision (Inter-day) study of TOLP

Sr. no.	Conc. (µg/ml)	Standard solution		Sample solution	
		Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
1	200	2203595.174	2204016.216	2204097.172	2204263.250
2	200	2203762.637	2203651.764	2203546.587	2203941.746
3	200	2203195.423	2203685.420	2203929.486	2203599.112
4	200	2203489.238	2203290.878	2203788.234	2204531.656
5	200	2203981.457	2204281.992	2203425.143	2204687.003
6	200	2203421.263	2204078.295	2203363.639	2203091.280
Mean		2203574.199	2203834.094	2203691.710	2204019.008
SD		274.185	358.845	293.259	601.937
%RSD		0.012	0.016	0.013	0.027

3.1.6 Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ of TOLP by the proposed method were found 0.172 µg/ml and 0.521 µg/ml, respectively. Figure 4(a) and 4(b) show the sensitivity of the current method.

Figure 4(b): Typical chromatograms of TOLP at LOQ concentration.



3.1.7 Reproducibility: 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 judged.

Table 5: Reproducibility study of TOLP (50 µg/ml)

Instrument 1	Instrument 2	Result of t test*	Inference
2300960.912±27899.614	2322036.197±30331.080	0.185	No significant difference

* At 95% confidence interval, (t-Tabulated = 2.45)

3.1.8 Robustness: The % of RSD of robustness testing under different altered conditions is given in Table 6, indicating that the current method is robust.

Table 6: Robustness evaluation of the proposed method.

Parameter	Conditions	Amount of TOLP added (µg/ml)	Amount of TOLP detected (Mean ± SD)	%RSD
Change in mobile phase composition	ACN : Buffer = 53:47	50	49.61 ± 0.47	0.95
	ACN : Buffer = 55:45	50	49.55 ± 0.01	0.02
	ACN : Buffer = 57:43	50	49.32 ± 0.39	0.79
Change in flow rate	0.9ml/min	50	49.92 ± 0.02	0.04
	1.0 ml/min	50	49.88 ± 0.01	0.02
	1.1 ml/min	50	49.67 ± 0.04	0.08

3.2 Determination of TOLP in marketed formulations: Two marketed formulations of TOLP were analyzed by proposed RP-HPLC method and the results of this analysis are shown in Table 7.

Table 7: Assay of Marketed Formulations of TOLP by proposed RP-HPLC method

Formulation (Tablet)	Actual concentration (µg/ml)	Amount obtained (µg/ml)	% TOLP
Brand 1 (Tolpidol®)	50	49.23	98.46
Brand 2 (Tolfree®)	50	49.70	99.40

Conclusion

The proposed RP-HPLC method for estimation of Tolperisone Hydrochloride in bulk and in tablet dosage form was found to be simple, accurate, precise reproducible and highly sensitive. The % recovery and specificity study show that the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time of 5 min leads to cost effective chromatographic method. The measured signals were shown to be precise, accurate and linear over the wide concentration range with a correlation coefficient around 0.999. The % RSD for all parameters were found to be less than two, which indicates the validity of method and assay result obtained by this method is in fair agreement. Therefore it seems to be suitable for the routine quality control analysis in analytical lab.

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