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Novel and validated first derivative UV Spectrophotometric method for the determination of Tolperisone hydrochloride in pharmaceutical preparations

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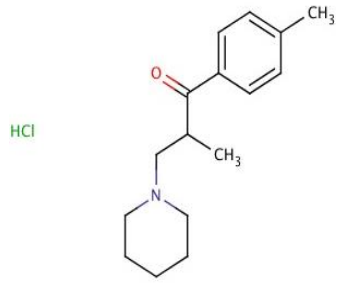
ABSTRACT

A validated first order derivative spectrophotometric method was developed for the quantification of Tolperisone in bulk and its dosage form which is simple, novel, sensitive, specific and rapid. The method is based on the conversion of zero order spectrums to first derivative mode at an absorption maximum of 260nm employing distilled water as the solvent. Beer's law followed in the concentration range of 10-50µg/ml. Accuracy of the method was indicated by recovery studies and ICH guidelines were followed for the validation of the proposed method. The results were found agreeable and repeatable. The method was applied successfully for the determination of Tolperisone in bulk drug and dosage form.

KEY WORDS: Tolperisone, First derivative, UV Spectrophotometric, UV determination

1. INTRODUCTION



Structure of Tolperisone Hydrochloride

Tolperisone HCl: Tolperisone is a piperidine derivative, which is centrally acting muscle relaxant. Chemically it is 2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one hydrochloride. Tolperisone is a centrally acting muscle relaxant launched for the treatment of severe and persistent back pain and paroxysmal symptoms of neurological cause. As a centrally acting muscle relaxant, Tolperisone blocks sodium channels and identified to possess significant effect on voltage- gated calcium channels (Internet). Only some methods have been reported so far in past studies for the determination of Tolperisone in its single component dosage forms. The methods reported are four HPLC methods (Koladia and Vaghela, 2012; Seema and Nandini, 2013; Chhalotiya, 2013; Mahaparale and Gonjari 2015), two UV spectrophotometric zero order methods (Koladia and Vaghela, 2012; Carolin, 2011) one visible spectrophotometric (Colorimetric) method (Sai Praveen, 2010) and one HPTLC method (Usmangani, 2013). No method by First derivative uv spectrophotometry erstwhile reported and there for the objective of the present study was to develop a simple, interference free and rapid first derivative spectrophotometric method which is helpful for routine analysis with comparatively similar accuracy and precision as reported in earlier methods.

2. MATERIALS AND METHODS

Materials: Shimadzu 1800 UV Spectrophotometer spectronic model with a pair of matched 1cm quartz sample cells was used for collection of data and data analysis. Distilled water was employed as a solvent for the drug substance. Commerical brands for the assay parameter were obtained from local market.

Methodology

Preparation of standard stock solution: The standard stock solution was prepared by weighing 25mg Tolperisone and transferring in to a 25ml volumetric flask. 10 ml distilled water was added in to this volumetric flask and dissolved completely. The volume was made up to the mark to give a solution containing 1000 µg/ml Tolperisone, using distilled water. A volume of 2.5ml from this solution was transferred to 25ml volumetric flask and the volume was made up to the mark with the distilled water to get a solution containing 100µg/ml of Tolperisone.

Determination of λ max: A volume of 2.5 ml of standard stock solution of Tolperisone was put into 25 ml volumetric flask and the volume was brought up to the mark to obtain the solution of concentration 10µg/ml, with distilled water as solvent. The solution was scanned in the UV range of 200nm to 400 nm and the λ max was found to be 260 nm. The spectrum of Tolperisone was recorded with N=5 in first derivative mode.

Stability of Drug in the chosen Solvent: The stability of Tolperisone in the selected solvent was determined by measuring the absorbance of the drug solution (20µg/ml) at different time interval. The absorbance was noted after every 5 minutes, up to 15 minutes the readings were stable.

Determination of Beer-Lambert's Range: Different volumes 2.5, 5, 7.5, 10, 12.5 ml from the standard stock solution of Tolperisone were transferred to five separate 25 ml volumetric flask and volume were adjusted with distilled water up to the mark to obtain concentrations 10, 20, 30, 40, 50 µg/ml respectively. These concentrations were selected because, the concentrations below and above this range were not linear when calibration curve was plotted. (Table 1, Figures 1 to 4)

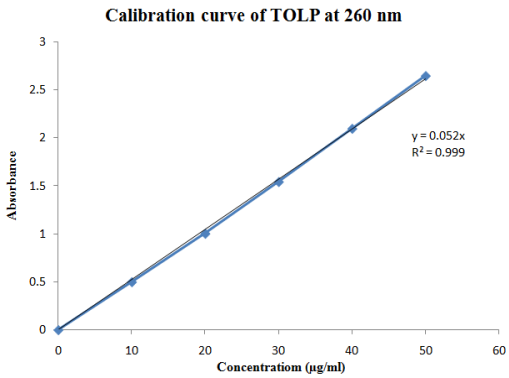


Figure.1. Calibration curve of Tolperisone at 260 nm

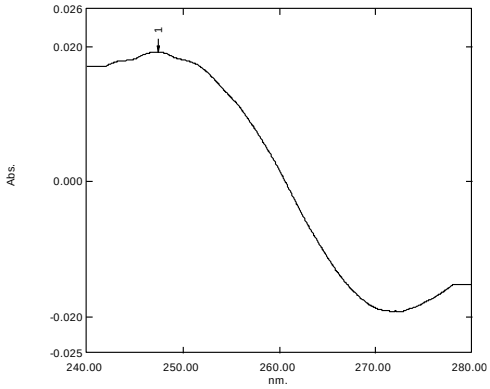


Figure.2. First derivative spectrum of Tolperisone 10 µg/ml

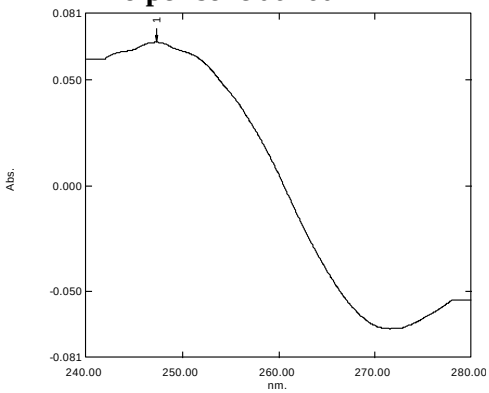


Figure.3. First derivative spectrum of Tolperisone 30 µg/ml

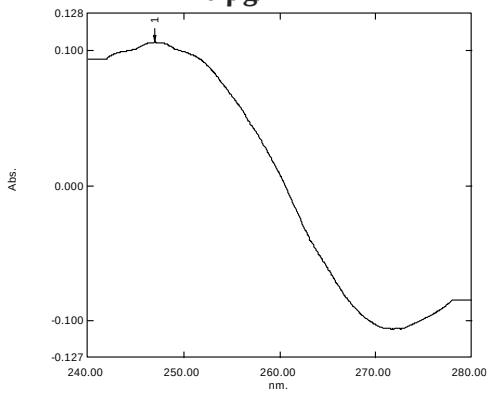


Figure.4. First derivative spectrum of Tolperisone 50 µg/ml

Table.1. Optimum Parameters of the Calibration Plot

Parameters	Tolperisone
Linearity range (µg/ml)	10-50
Slope	0.0539
Intercept	-0.0585
Regression coefficient(r^2)	0.999

Estimation of Tolperisone in bulk drug: In order to see the feasibility of proposed method for the determination of Tolperisone in marketed pharmaceutical formulations, the method was first employed for estimation of Tolperisone in standard bulk sample.

An accurately weighed amount of 25 mg of Tolperisone was put into 25 ml volumetric flask, dissolved in Distilled water by vigorous shaking and volume was made up to the mark using the same solvent. Appropriate aliquot 2.5 ml was transferred to 25 ml volumetric flask and volume was made up to mark with distilled water to get a concentration 100µg/ml. From that appropriate aliquot 5 ml was transferred to 25 ml volumetric flask and volume adjusted to mark with the same solvent to get the concentration 20µg/ml. The absorbance of the solution was recorded at 260 nm in first derivative mode at N=5 against reagent blank and results were satisfactory.

Validation of proposed method

Application of proposed method for analysis of tablet formulation: For analysis of commercial formulation; 10 tablets of marketed brand of Tolperisone were crushed and weighed powdered tablet equivalent to 100mg of Tolperisone was taken and dissolved in distilled water to get 100µg/ml stock solution. Then the solution was diluted to get final concentration of 20µg/ml solution using distilled water. The absorbance at 260 nm was measured using first derivative spectrum with N=5. Results are shown in Table 2.

Table.2. Assay of Tolperisone in Tablet formulation (Commercial Brand)

Amount Taken (mg/tab)	Amount found (mg/tab)	Amount found (%)
450	445.9	99.10
450	447.1	99.36
450	450.5	100.13
450	449.8	99.96
450	453.6	100.81
	Mean	99.87
	SD	0.6731
	CV	0.4530

Accuracy (Recovery Test): Recovery experiments were employed to find out the accuracy. The recovery testing was completed by the addition of known amounts of pure drug to tablet samples. The recovery was performed at three different levels, i.e. 80%, 100%, and 120 % of Tolperisone standard concentration. The recovery samples were prepared as in previously mentioned procedure for tablet assay. Three samples were prepared for every recovery level. The solutions were analyzed, and the percentage recoveries were calculated by using following formula and data is tabulated in Table 3.

$$\%\ \text{Recovery} = \frac{\text{Observed amount of compound in sample}}{\text{Amount of all compound present in sample}} \times 100$$

Table.3. Results of accuracy parameter of Tolperisone

Level of % Recovery	Amount present (µg/ml)	Amount of standard added (µg/ml)	Total amount recovered (µg/ml)	% Recovery	% mean Recovery	SD	CV
80	450	360	805.46	99.440	99.628	0.5140	0.2642
80	450	360	803.80	99.235			
80	450	360	811.70	100.210			
100	450	450	904.23	100.471	99.852	0.5938	0.3526
100	450	450	893.58	99.287			
100	450	450	898.18	99.798			
120	450	540	992.55	100.258	99.753	0.5751	0.3307
120	450	540	981.35	99.127			
120	450	540	988.75	99.874			

Precision: Precision of the method was examined by carrying out assays by three independent procedures on Tolperisone tablet samples. The intermediate precision of the method was assessed by four different analysts, systems in the same laboratory. The Assay values obtained by four analysts were given in Table 4.

Table.4. Determination of Precision of Tolperisone

Sample Number	Assay of Tolperisone as % of labeled amount			
	Analyst- 1	Analyst-2	Analyst-3	Analyst-4
1	99.14	99.69	100.39	99.43
2	99.75	99.50	99.05	100.92
3	100.35	100.71	99.51	99.51
4	99.02	99.43	99.80	99.23
5	99.88	99.18	99.79	100.33
6	99.37	99.81	100.12	99.97
Mean	99.58	99.72	99.77	99.89
S.D.	0.5025	0.5316	0.4677	0.6406
CV	0.2525	0.2826	0.2187	0.4104

3. RESULTS AND DISCUSSION

The standard solutions of Tolperisone in distilled water (10µg/ml each) subjected to a scan 200 nm to 400 nm at zero order and the first derivative spectra were taken at N=5 using Shimadzu 1800 UV-Visible spectrophotometer spectronic model. The λ max was found to be at 260 nm. The calibration curve of Tolperisone was found to be linear at 260 nm. Beer's law followed in the concentration range of 10-50 µg/ml.

Validation of the newly developed method was determined as per the international guidelines and parameters. The novel methodology for the quantitative investigation of Tolperisone was subjected to different validation parameters like selectivity and specificity in presence of formulation additives and excepients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was

optimized, accuracy was proved by recovery studies at different concentration levels, precision was established through different analyst studies.

With the aim of determining the practicability of the developed technique for the assessment of commercially available brands of medicinal formulations, the technique was initially attempted on bulk drugs and concentrations were estimated. Then the technique was subjected to the assay of tablets in marketed drug samples and satisfactory results were attained within the acceptable limits as per the content of the label claim for Tolperisone.

The technique was validated by principles of ICH guidelines for various parameters including specificity, linearity, accuracy, precision- repeatability and the results were found to be reasonable with lower standard deviation and coefficient of variation values within the acceptable limits for Tolperisone in their bulk and dosage forms i.e. marketed tablet formulations for their First derivatization UV-spectrophotometric estimation. The method showed specificity in presence of formulation additives, because there was no interfering from the tablets formulation additives. The method was linear, with low deviation values and the regression equation was calculated by the method of least squares. The method was accurate as indicated by satisfactory recovery studies at different level of confidence. Studies on intermediate precision were carried out by different analysts and the results were found to be all right demonstrating that, the process was reproducible. The scheme was not inclined to small change in the method parameters, because the data obtained were reproducible in different temperature conditions applied at the time of determination of Tolperisone with very negligible deviations under the conditions employed.

The described method offers precise and accurate results for the quantitization of Tolperisone in its bulk drug samples and tablet formulations and applied without any difficulty for the regular determinations. The method is also simple, rapid and economical method which gives reproducible results on the instrument used. The reported method is an economical method in which only distilled water is employed as the solvent and does not necessitate the use of costly reagents. This proposed method is competent of being used for the quantification of Tolperisone in its bulk and tablet dose forms devoid of the interfering of additives with a significant and comparative correctness and exactness with the reported methods. The newly reported method has the advantages over the previously reported methods because, present methods is economical.

The percentage standard deviation values show that this novel method provides acceptable variation of Tolperisone. The low percentages standard deviation of proposed technique is within the acceptable limits for Tolperisone shows the competence of the technique to stay unchanged by minute and purposeful alterations in the system parameters and assures its consistency in regular routine application.

4. CONCLUSION

The results and the statistical parameters demonstrate that the proposed First derivative UV spectrophotometric method for Tolperisone is simple, rapid, specific, accurate and precise. The most prominent attribute of spectrophotometric methods are their simplicity and rapidity. Result of validation parameters demonstrated that this new analytical method for determination of Tolperisone is appropriate for its projected purpose and meets the criteria defined in ICH Q2/B. This new method is useful for routine analysis of Tolperisone in its single component dosage forms without interference of impurities.

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