SHORT NOTES

DEVELOPMENT AND VALIDATION OF DIFFERENCE SPECTROSCOPIC METHOD FOR THE ESTIMATION OF TOLPERISONE HCL IN BULK AND PHARMACEUTICAL DOSAGE FORM

ABSTRACT

Difference spectroscopic method is based on the principle that tolperisone hydrochloride can exhibit two different absorption spectra in basic and acidic medium. A stock solution (1 mg/mL) was prepared with distilled water. Further dilution was made by using 0.1 N sodium hydroxide and 0.1 N hydrochloric acid separately. The maxima and minima in the difference spectra of tolperisone hydrochloride were at 231nm and 263nm, respectively. Difference in absorbance between these maxima and minima was calculated to find out the amplitude. This amplitude was plotted against concentration. Linearity was found in the concentration range of 10-50µg/mL. The LOD and LOQ of tolperisone hydrochloride were found to be 0.064µg/mL and 0.196µg/mL respectively. The percentage recovery study of the drug for the proposed method was found in the range of 99.31 - 99.94% for tolperisone hydrochloride. The proposed method is recommended for routine analysis since it is rapid, simple, precise and accurate.

Keywords: Difference Spectroscopic Method, Amplitude, Linearity, Recovery Studies.

INTRODUCTION

Tolperisone hydrochloride, chemically 2- methyl-1- (4 –methyl phenyl) – 3- (1- piperidyl) propane-1 one, is a piperidine derivative and the structure is shown in Fig.1. It is a centrally acting muscle relaxant which is used in the treatment of different pathological conditions like low back pain syndrome, pain-central stoke spasticity, neuropathyrism, trapezitis and periarthrits[1]. Tolperisone hydrochloride is official in Japanese Pharmacopoeia[2]. The literature survey revealed that there are some analytical methods reported for tolperosine hydrochloride either individually like UV-visible spectrophotometric method[3,4], HPTLC[5], or in combination with other drugs by RP-HPLC[6-8] and also reported on biological fluids[9]. The present work is an attempt to develop a simple, accurate, precise and validated difference spectroscopic method for the estimation of tolperisone hydrochloride in bulk and tablet dosage form.

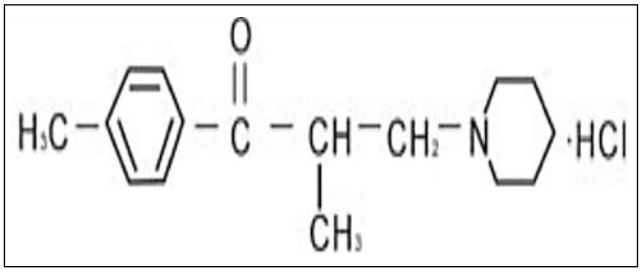

Fig. 1: Structure of tolperisone hydrochloride

MATERIALS AND METHODS

Instrumentation

An UV – Visible spectroscopy instrument of model SL218 (ELICO) which is a double beam instrument with 1.5 cm slit, matched with 1cm path length quartz cells were used for this experiment. Digital balance (Shimadzu AX 200) was employed for the estimation.

Chemicals and Reagent

Tolperisone hydrochloride reference standard was kindly provided by Themis Medicare Ltd, Vapi. The purity of reference standard was 99.87%w/w. Tablet formulations containing tolperisone hydrochloride (TOLIFAST 150mg) of Lupin Pharmaceuticals Ltd was used for this estimation.

Table I: Data from the analysis of tablet formulation (n=3)

Tablet	Label Claim (mg/ tab)	Amount Found (mg/tab)	%Assay ± SD
Tolifast	150.00	149.06	99.37 % ± 0.72

Table II: Data of Optical Characteristics for Tolperisone HCl

Parameters		Observed Value
Wavelength		[a]231 nm, [b]263 nm
Beer's Law Limit (µg/mL)		10-50 µg/mL
Molar absorptivity (lit./mole/cm)		0.451×10^4
Sandell's sensitivity (µg cm^{-2}/0.001 absorbance unit)		0.0625
Correlation coefficient (r^2)		0.9992
Regression equation y = mx + c	Slope (m)	0.017
	Intercept (c)	0.0226

Table III: Result of Intermediate Precision (n=6)

Intraday precision Mean ± SD	Interday Precision Mean ± SD	%R.S.D.	
		Intraday Precision	Interday Precision
29.92 ± 0.069	29.94 ± 0.102	0.34	0.51

Table IV: Recovery data for Tolperisone HCl from Tablet formulation (n=3)

% Level	Amount of Drug in Sample (µg/ml)	Amount of Std. drug added (µg/ml)	Mean % Recovered ± SD
50	20	10	99.31 ± 0.12
100	20	20	99.94 ± 1.66
150	20	30	99.88 ± 0.21

Table V: Data of validation parameters for tolperisone hydrochloride

Parameters	Observed Value
Linearity Range	10-50 µg/mL
Intraday Precision(%RSD)	0.34%
Interday Precision(%RSD)	0.51%
Accuracy	99.31-99.94%
LOD	0.064 µg/mL
LOQ	0.196 µg/mL

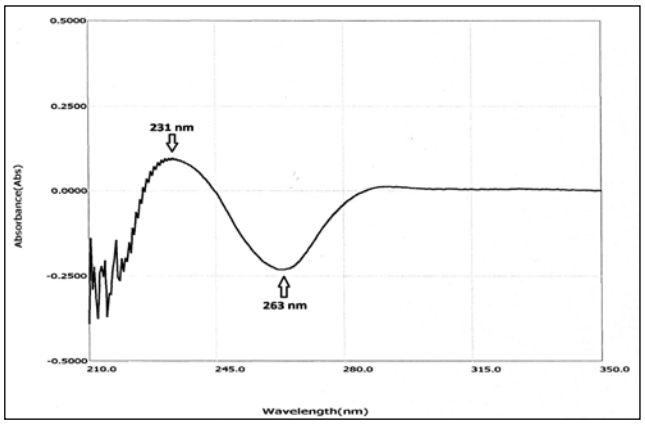

Fig. 2: Difference spectrum of tolperisone hydrochloride (30 µg/ml)

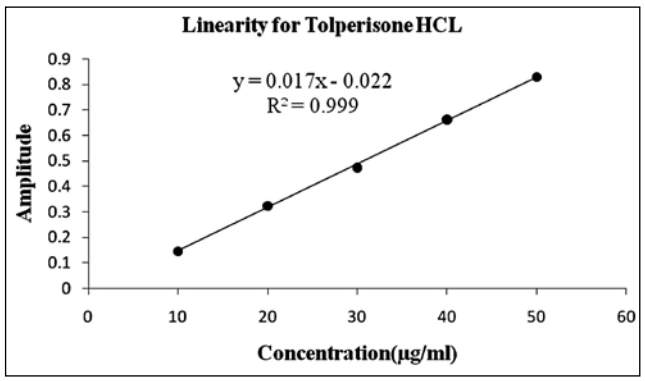

Fig. 3: Calibration curve of tolperisone hydrochloride

Preparation of Standard Stock Solution

Accurately weighed quantity of Tolperisone HCL 50 mg was transferred into 50 mL volumetric flask dissolved it and diluted up to mark with distilled water. This gave a stock solution having strength of 1000 µg/mL.

Preparation of working standard solution

The standard solution was further diluted with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide separately to get the concentration of 100 µg/mL

Determination of wavelength for measurement

From the working standard solution, 10 µg/mL solutions were prepared separately by using 0.1 N Hydrochloric acid and 0.1 N Sodium hydroxide. Solution was scanned between 200-400 nm. Wavelengths were selected from the difference spectra of tolperisone hydrochloride.

Calibration curve for Tolperisone HCl

Different aliquots were taken from their working standards and diluted with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide separately to prepare a series of concentrations from 10-50 µg/ml as reference and test solutions, respectively. Difference spectrum was recorded by placing tolperisone hydrochloride in 0.1 N hydrochloric acid in reference cell and 0.1 N Sodium hydroxide in sample cell. Difference in absorbance between maxima and minima was calculated to find out the amplitude. The calibration curve was prepared by plotting amplitude versus concentration.

Estimation of Tolperisone HCl in tablet dosage form

Twenty tablets were weighed and their average net weight was calculated. The tablets were crushed and the powder was made to a fine powder. The powder equivalent to 50 mg of tolperisone hydrochloride was weighed and transferred in to 50 mL volumetric flask. Dissolved in distilled water and made up to the volume with the same. The solution was filtered through Whatman filter paper No.41. From the stock solution, 30 µg/mL solutions were prepared separately by using 0.1 N hydrochloride acid and 0.1 N sodium hydroxide. The amplitude was calculated by measuring the absorbance of the equimolar concentrations at maxima and minima in the difference spectrum. The amount of tolperisone hydrochloride was calculated.

METHOD VALIDATION

Linearity and Range

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 10 – 50 µg/ml for tolperisone hydrochloride. Plot the calibration curve of amplitude v/s concentration and determine correlation coefficient and regression line equations for tolperisone hydrochloride.

Accuracy

Preparation of Sample Solution

Twenty tablets were powdered. Powder equivalent to 50 mg of tolperisone hydrochloride was weighed and transferred into 50 mL of volumetric flask, sonicate it for 10 minutes and diluted up to mark with distilled water. The solution was filtered using Whatman filter paper no.41 and first few drops of filtrate were discarded. From this solution made 100 µg/mL of tolperisone hydrochloride. To 2 mL of the above solution, increasing aliquots of working standard solution (1, 2, 3 ml of 100 µg/ml of tolperisone hydrochloride working std. solution) was added and diluted to 10 ml with 0.1 N hydrochloric acid and same series was made by using 0.1 N Sodium Hydroxide. Absorbance of solution was measured at selected wavelengths for tolperisone hydrochloride. The amount of tolperisone hydrochloride was calculated at each level and % recovery was computed.

PRECISION

(I) Intraday Precision

Solutions containing 30 µg/mL tolperisone hydrochloride was analyzed three times on the same day and %R.S.D were calculated.

(II) Interday Precision

Solutions containing 30 µg/mL tolperisone hydrochloride was analyzed on three times in different days and %R.S.D were calculated.

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

The LOD and LOQ of tolperisone hydrochloride by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10 σ /S respectively, where S is the slope of the calibration curve and σ is the standard deviation of response.

RESULTS AND DISCUSSION

The solubility of tolperisone hydrochloride was studied and methanol was selected as a choice of solvent. tolperisone hydrochloride showed well defined maxima at 231nm and minima at 263nm, therefore these wavelengths were considered for development of difference spectroscopic method (Fig. 2). Linearity was in the concentration range of 10-50 µg/mL for tolperisone hydrochloride (Fig. 2). Coefficient of correlation for tolperisone hydrochloride were found to be 0.9992. The values of correlation coefficient suggest the level of precision of the method. Drug content in tablet (amount present) was directly found from regression equation. Standard deviation was calculated and is given in Table I. Percentage estimation in tablet dosage form was 99.37 % ± 0.72 for tolperisone hydrochloride. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Linear regression equations (intercepts and slopes) for tolperisone hydrochloride was established. The high values of the correlation coefficients and the values of y-intercepts close to zero indicate the good linearity of the calibrations. The values of slope, intercept and correlation coefficient values are given in Table II. Limit of detection (LOD) and limit of quantitation (LOQ) was found to be 0.064 and 0.196 µg/mL. In intermediate precision, a study carried out by the same analyst working on three times in the same day and three consecutive days indicated the %RSD of 0.34 and 0.51 for intraday and interday analysis, respectively. Both the percentage RSD values were of below 2%, indicated that the intermediate precision was confirmed (Table III). To study the accuracy, recovery experiment was carried out by standard addition. The recovery of added standard was calculated at different concentration levels. From the total amount of drug found, the percentage recovery was calculated which was between 99.31-99.94%, indicating that the method was accurate (Table IV).

CONCLUSION

The proposed method is simple, accurate, precise and sensitive for the estimation of tolperisone hydrochloride in bulk and in tablet dosage forms. The method is economical, rapid and do not require any sophisticated instruments contrast to chromatographic method. Hence it can be effectively applied for the routine analysis of tolperisone hydrochloride in bulk and in tablet dosage forms.

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