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Research Article

Spectrophotometric determination of Tolperisone using 2, 4-dinitrophenylhydrazine reagent

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ABSTRACT

A simple, novel, sensitive, and specific spectrophotometric method was developed and validated for the determination of Tolperisone in bulk and its dosage forms. The proposed method was based on the interaction of the drug with 2,4-dinitrophenylhydrazine in the presence of an acid catalyst, followed by treatment with a methanolic solution of potassium hydroxide; an intensely colored chromogen was formed that was measured in dimethyl formamide as the diluting solvent at 520 nm. All variables affecting the development of the measured chromogen were studied and optimized. Beer's law was obeyed in the concentration ranges of 2.5-15 µg/ml with good correlation coefficient of 0.9995. The limit of detection (LOD) and limit of Quantification (LOQ) for this method are 0.344 and 1.043 µg/ml respectively, and the relative standard deviation of intra-day precision was 1.10% and inter-day precision was 1.42%. The proposed method was applied successfully for the determination of Tolperisone in pure bulk form and in tablets without interference from commonly encountered additives.

Keywords: Tolperisone; 2, 4 Dinitrophenylhydrazine; Spectrophotometry.

INTRODUCTION

Tolperisone (Martindale 1993), chemically 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one is a piperidine derivative (Rumiko Tanaka et al., 2007). It is a centrally acting muscle relaxant (Hofer D et al., 2006, Kocsis P et al., 2005) which is used in the treatment of different pathological conditions like multiocular sclerosis, myelopathy, encephalomyelitis, Spondylosis, Spondylarthrosis, Cervical and lumbar syndromes,Arthrosis of the large joints, Obliterating atherosclerosis of the extremity vessels, Diabetical angiopathy, Thromboangiitis obliterans,Raynaud's syndrome (Gayraud M., 2007). A number of methods such as HPLC (Bernadett Stiedl et al., 2009, Saisunee Liawruangrath et al., 2001), LCMS (Min Kyo Jeoung et al., 2007) were reported for the quantitative estimation of tolperisone. Literature survey reveals that no spectrophotometric methods have been reported for the estimation of this drug in bulk form and in its formulations. The present work is an attempt to develop a rapid and sensitive method for the colorimetric estimation by 2, 4 Dinitrophenylhydrazine [2, 4 DNP] (Osama H.Abdelmageed et al., 2007) . 2, 4 DNP also known as Brady's Reagent has been used for the characterization of aldehydes and ketones by hydrazone formation. We report here its application in the estimation of Tolperisone (ketone group).The developed method is simple, accurate and applicable for their determination in pharmaceutical formulations.

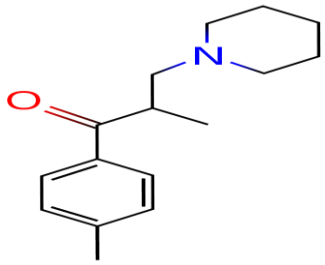

Figure 1: Structure of Tolperisone

EXPERIMENTAL

Apparatus

A Systronics Model 2201 UV-Visible Spectrophotometer with matched 1 cm Quartz cells were used throughout the study for all measurements. A thermostatically controlled water bath was used.

Chemicals and Reagents

All chemicals of analytical grade were used.

1. 0.005M 2,4-Dinitro Phenyl Hydrazine Reagent: The reagent was freshly prepared by dissolving 0.1 g of 2,4-Dinitro Phenyl Hydrazine in a mixture of 10 ml of methanol and 0.5 ml of conc.HCl and diluting the resultant mixture to 100 ml with methanol.

2. 0.1786 M KOH solution: 0.1786 M KOH solution was prepared by dissolving 10 g of KOH in 20 ml distilled

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water and diluting to 100 ml with methanol. A suitable aliquot was taken (1 in 10 dilution) to obtain the 0.1786 M solution.

3. Preparation of Standard Solution: A stock solution of 1 mg/ml was prepared in methanol. This solution is diluted to obtain the required concentration (100 µg/ml).

4. Preparation of Sample solution: 20 tablets were weighed and powdered. An amount equivalent to 100 mg of Tolperisone was weighed and transferred to a 100 ml flask. To it 50 ml of methanol was added and sonicated for 15 minutes. The solution was filtered and made upto 100 ml with methanol. This solution was suitably diluted to obtain the required concentration.

PROCEDURE

Aliquots of working standard solution of Tolperisone 0.25-1.5 ml (100 µg/ml) were transferred into a series of 10 ml calibrated test tubes. To this 2 ml of 2,4-DNP reagent was added followed by 1 drop of conc.HCl. The mixture was placed on a boiling water bath for evaporation of the solvent to almost dryness. The residue was cooled to room temperature and 3 ml of 0.1786 M KOH solution was added. The contents of the tubes were mixed thoroughly and allowed to stand for 30 minutes with occasional shaking at room temperature. The contents of the tubes were transferred quantitatively to a 10 ml volumetric flask and made upto the volume with Dimethyl formamide. The contents in the flask were mixed well and the pink colored chromogen was measured spectrophotometrically at 520 nm against a reagent blank.

Optimization of experimental conditions

Effect of reaction time and temperature

The optimum reaction time and temperature were determined by following the color developments at different temperatures ranging from 50-100 °C for different time periods when the suggested procedures were used. Satisfactory results for maximum color intensity and reproducible (λ_{max}) values were obtained only after boiling the solution at 100 °C for 5 min, beginning when the solution began to boil and continuing it until the solution almost evaporates.

Effect of volumes of reagent and acid catalyst

Different volumes of prepared 2,4-DNP reagent from 0.5 – 4 ml and in parallel with volume of conc.HCl from about 1 drop equivalent to 0.05 ml -0.5 ml were studied with the mentioned procedure. Optimum color intensity and reproducible values (λ_{max}) were obtained with 2 ml of reagent and 0.05 ml of conc.HCl. Excess acid should not be used inorder to avoid heavier precipitation of Potassium chloride upon the addition of base.

Effect of volume and concentration of Base

It was observed that maximum intensity, better resolution with sharp peaks and reproducible values (λ_{max}) are attained with 2.5-3.5 ml of 0.1786 M alcoholic KOH. With much higher concentration of base no satisfactory results were obtained because the color of the blank remained dark for a long time and the product showed slight turbidity after dilution with Dimethyl Formamide.

Effect of waiting time before dilution with diluting solvent

Upon addition of the base a dark greenish solution was formed, the color of the blank changed slightly to pale yellow but the color of the sample turned pink. Thus the time required for the stable intensity with maximum absorbance was studied with different intervals ranging from immediate measurement to a waiting period of 45 minutes. It was found that a waiting period of 25 minutes is essential for Tolperisone. Thus 30 minutes is chosen for subsequent testing.

Effect of Diluting solvent

Solvents with different dielectric constants were tried such as DMF, acetonitrile, chloroform, 1, 2 dichloromethane, cyclohexane, methanol, water and toluene. Satisfactory results were obtained only with DMF, followed by acetonitrile. Results indicated the importance of using a solvent of intermediate polarity. Thus DMF was chosen for the study.

Validation of the Proposed Method

The validation of the developed spectrophotometric method was carried out as per the ICH guidelines (ICH Q2B 1996). According to the ICH guidelines the following parameters are evaluated.

Linearity

Under the optimized experimental conditions, the calibration curve for Tolperisone was constructed by analyzing a series of standard solutions of the drug. The regression equations for the results were derived by using the least squares method. The Beers law plot was linear (2.5 – 15 µg/ml) with very small intercept and good correlation value (0.9995). The LOD and LOQ values were found to be 0.344 and 1.043 µg/ml respectively. The calculated Molar absorptivity indicated that the suggested procedure is highly sensitive (Table-1).

Repeatability

The precision of the method was checked by replicate analysis of 6 separate sample solutions of the drug at three different levels. Similarly the inter-day precision was repeated at the same concentration levels for 3 consecutive days. The intraday precision and inter-day precision values were found to be within the limits indicating that the method developed was precise (Table-2).

Table 1: Optical characteristics and statistical data of the regression equation for the reaction of the proposed method

Parameters	Optical characteristic
Color	Pink
λ_{max} (nm)	520
Beers law limits (µg/ml)	2.5 - 15
Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.0128
Molar Absorptivity (litre mole^{-1} cm^{-1})	2.4 x 10^4
Limit of Detection (LOD)	0.344
Limit of Quantification (LOQ)	1.043
Regression Equation	0.0565x+0.0497
Slope	0.0565
Intercept	0.0497
Correlation intercept	0.9995

Table 2: Intra-day and inter-day precision data

Drug	Amount taken (µg/ml)	Intra-Day Precision* (%)	Inter-Day Precision** (%)
Tolperisone	5	1.06	1.38
	7.5	1.22	1.46
	10	1.02	1.42

*Mean of 6 determinations,
**Mean of 6 determinations performed over a period of 3 days

Accuracy

The accuracy of the proposed method was also confirmed through recovery studies using the method of standard additions. Results indicated good recoveries which reflect the selectivity of the extraction procedure for Tolperisone from the commonly encountered common excipients and additives. Therefore the proposed method can be considered specific for the determination of Tolperisone in commercially dosage forms (Table-3).

Table 3: Estimation of Tolperisone in Pharmaceutical Formulations and Recovery studies

Formulations (Tablets)	Labelled amount (mg)	Amount found* by proposed method	% recovery** by proposed method
Tablet 1	100	98.72	98.81
Tablet 2	100	98.16	99.67
Tablet 3	150	146.9	99.18
Tablet 4	150	147.24	99.25

***** Average of six determinations
******Recovery of amount added to the pharmaceutical formulation (Average of three determinations)

RESULTS AND DISCUSSION

Selection of this reagent was based on the higher reactivity of 2, 4-DNP compared to other hydrazine derivatives and the presence of strong chromophore group (Y.R. Sharma 2002) in its structure that enables its use for the colorimetric determination of several aldehydes and ketones. In addition to being specific for carbonyl groups 2, 4-DNP has advantages over other reagents such as dyes in ion-pair spectrophotometry, as not much care is needed regarding the pH of the reaction and there is no need to extract the product formed. Also the hydrazone and hydrazide products are more red shifted than those of other methods.Also the stability of the colored product can be accounted for by the fact that the reaction is stable as the potassium salt.

The optical characteristics such as beers law limits, Sandell's sensitivity, and molar extinction coefficient, percent range of error (0.05 and 0.01 confidence limits) were calculated and results are summarized in Table 1. The intraday and Inter-day Precision values were summarized in Table-2. The values obtained for the determination of Tolperisone in Pharmaceutical formulations (Tablets) by the proposed methods are presented in Table 3. Studies reveal that the common excipients and other additives usually present in the Tablets did not interference in the proposed methods.

CONCLUSIONS

A validated simple, sensitive, precise and specific spectrophotometric method is described for the determination of Tolperisone in Bulk drugs and in Tablet dosage forms. The proposed method is of great value in quality control determinations of Tolperisone 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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