Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2011, 3 (5):13-19
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Simple validated isocratic RP –HPLC method for estimation of Tolperisone in bulk and pharmaceutical dosage form

Murali. M[#] and Satyanarayana. P. V. V [*]

[#]*R.V.R & J.C. College of Engineering, Guntur, A.P., India*
[*]*Acharya Nagarjuna University, Guntur, A.P., India*

ABSTRACT

A simple, rapid reverse phase high performance liquid chromatographic method has been developed and validated for estimation of Tolperisone in pharmaceutical dosage form. The estimation was carried out on Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter column with a mixture of methanol: acetonitrile in the ratio of 90:10:(v/v) as mobile phase. UV detection was performed at 232 nm. The method was validated for linearity, accuracy, precision and specificity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 2.48 min. for Tolperisone and total run time was 5 min. at a flow rate of 1.0 ml/min. The calibration curve was linear over the concentration range of 40-100 ppm for Tolperisone. The LOD and LOQ values were found to be 0.5 and 3 ppm respectively. The high percentage of recovery confirms the suitability of the method for the estimation of Tolperisone in pharmaceutical dosage form.

Keywords: Tolperisone, RP-HPLC, Validation, tablet dosage form.

INTRODUCTION

Tolperisone a central muscle relaxant suitable for cerebral arteriosclerosis and for treating extrapiramidal movement disorders [1] is 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one, a piperidine derivative. Tolperisone has the unique property of mediating muscle relaxation without concomitant sedation and it does not cause inco-ordination, weakness and mental confusion or withdrawal phenomena, in contrast to other muscle relaxants. Its molecular formula and molecular weight are $C_{16}H_{23}NO.HCl$ and 281.83 respectively. Tolperisone HCl is

extremely water soluble, it is more stable in acidic medium (pH < 4.5), because it is disposed to decomposition in aqueous solution, which is faster at higher pH [2-4].

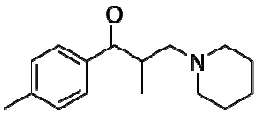


Figure 1: Structure of Tolperisone

Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of tolperisone which includes RP-HPLC[5-7], HPTLC[8], and UV-Visible Spectrophotometry [9-12]. Most of the analytical methods carried out by RP-HPLC to determine tolperisone found in the literature are aimed at quantifying tolperisone in biological fluids [13-14]. The target of this study is to develop a new, simple and fast analytical method by RP-HPLC to quantify tolperisone in bulk and its pharmaceutical dosage forms. This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines such as specificity, linearity, range, accuracy, precision, robustness to achieve an analytical method with acceptable characteristics of suitability, reliability and feasibility.

MATERIALS AND METHODS

Chemicals and reagents

Pure sample of Tolperisone was obtained from Shiwa Chemicals. Acetonitrile and methanol (HPLC grade) was procured from E. Merck, Mumbai (India).

Instrumentation and Chromatographic condition

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Inertsil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with methanol: acetonitrile 90:10 (V/V) was selected with a flow rate of 1.0 ml/min.The detection wavelength was set at 232 nm with a runtime of 5 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of standard Solution

Accurately weighed 100 mg of Tolperisone working standard was transferred into 100 ml volumetric flask, dissolved and volume was made up to the mark with methanol solution. The final stock solution contained 1000 ppm of Tolperisone. 10 ml of Tolperisone stock solution was transferred to a 100 ml clean volumetric flask and the volume was made up with diluting agent methanol and mixed well. The solution was then filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. 20 µL of final solution (100 ppm) was injected into the HPLC system and chromatograms were recorded.

Method development

The chromatographic condition was analysed with a view to develop a stability-indicating assay method for Tolperisone in pharmaceutical dosage form. Detection was performed at 232 nm which was based on UV scan of sample. Using Inertsil ODS C-18 column different mobile phase ratios of methanol and acetonitrile were run but the most selective peak was arrived by using them in the ratio of 90:10 respectively. The final chromatographic system optimized is shown in Table1.

Table 1: Optimized chromatographic condition of Tolperisone

Test	Condition
Mobile Phase	Methanol : Acetonitrile (90:10(v/v)), Isocratic
Diluent	Methanol
Column	Inertsil ODS C-18 (250 × 4.6 mm),5µm particle size
Column Oven	30°C
Flow rate	1.0 ml/min
Detector	UV at 232 nm
Injection Volume	20 µL
Run time	5 minutes
Pump pressure	9 psi

RESULTS AND DISCUSSION

Validation of the method

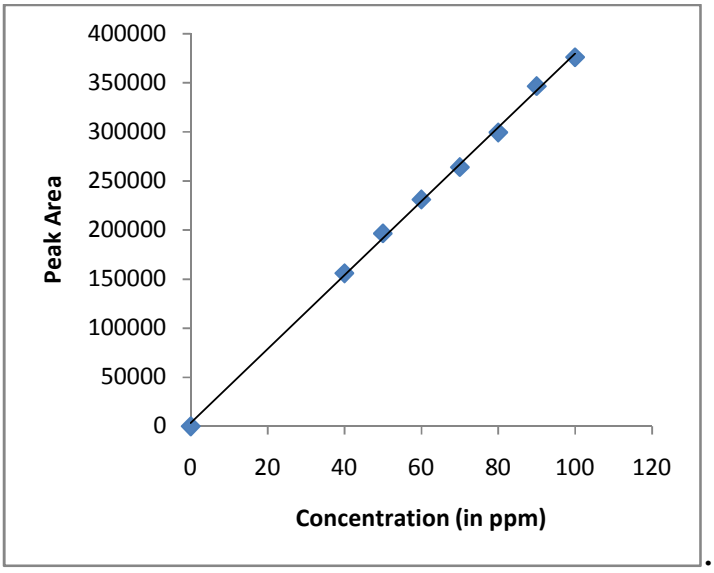


Fig. 2: Calibration curve of Tolperisone as per the proposed RP-HPLC method

Linearity

The linearity of the response for Tolperisone assay method was determined by preparing and injecting standard solutions with concentrations of 40 - 100 ppm Tolperisone. The linear

regression data for the calibration curves (Fig.2) indicate that the response is linear over the concentration range studied with correlation coefficient (r^2) value as 0.998. The values of slope and intercept were 3760 and 3392 respectively (Table 2). The standard deviation of y-intercept of regression line was determined and kept in following equation for the determination of detection limit and quantization limit. Detection limit= 3.3 σ/s; quantization limit = 10 σ/s, where σ is the standard deviation of y –intercept and s is the slope of the calibration curve.

Table 2: Regression analysis of the Calibration Curve

Parameters	Values
Calibration range	40-100 ppm
Regression equation	y = 3760 x + 3392
Slope(b)	3760
Intercept(a)	3392
Correlation coefficient(r^2)	0.998

Precision

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from five replicate injections of freshly prepared tolperisone test solution in the same equipment at a concentration value of 80 ppm on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the Tolperisone was determined and precision was reported as %RSD.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy is performed at three levels 25%, 50%, 75% by standard addition method. Data for recovery was obtained at three different concentrations of tolperisone standard.

Limit of detection (LOD) and limit of quantization (LOQ)

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guideline. For tolperisone, LOD was found to be 0.5 and LOQ was found to be 3.0 ppm.

Table 3: System suitability parameter for Tolperisone

Parameters	Values
λ max (nm)	232
Beer's law limit (ppm)	40-100
Correlation coefficient	0.998
Retention time	2.48 min
Theoretical plates	8084.1
Tailing factor	1.52
Limit of detection (ppm)	0.5
Limit of quantification (ppm)	3.0

Specificity

The specificity of the method was determined by comparing test results obtained from analyses of sample solution containing excuse ingredients (excipients) with that of test results those obtained from standard drug.

Robustness

Robustness of the method was determined by analyzing standard solution at normal operating condition and also by changing some operating analytical condition such as flow rate and mobile phase ratio. System suitability test was applied at each of above mentioned condition and results of test indicate robustness of method.

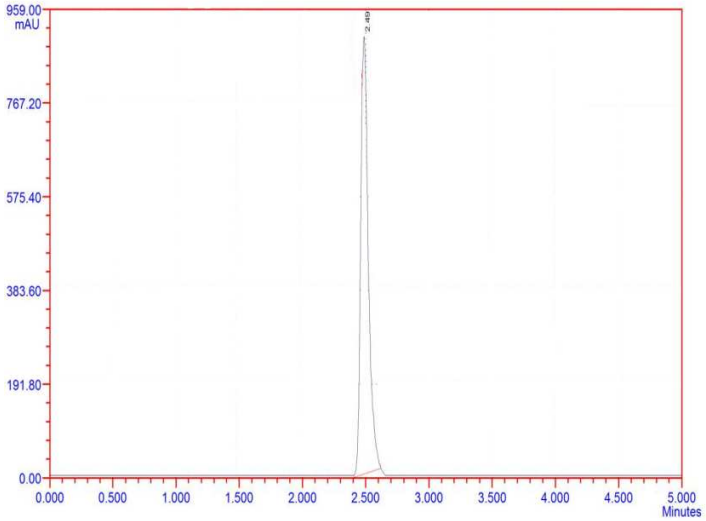


Fig. 2: A typical RP-HPLC chromatogram of Tolperisone

Table 4: Summary of validation parameters

Validation Parameter	Result
Linearity range	40-100 ppm
Target concentration	40 ppm
Precision	
System precision	0.035
Intermediate precision	0.102
Accuracy (% recovery)	98.57-101.35
LOD (ppm)	0.5
LOQ (ppm)	3.0
Specificity	No excipients interference
Robustness	Robust

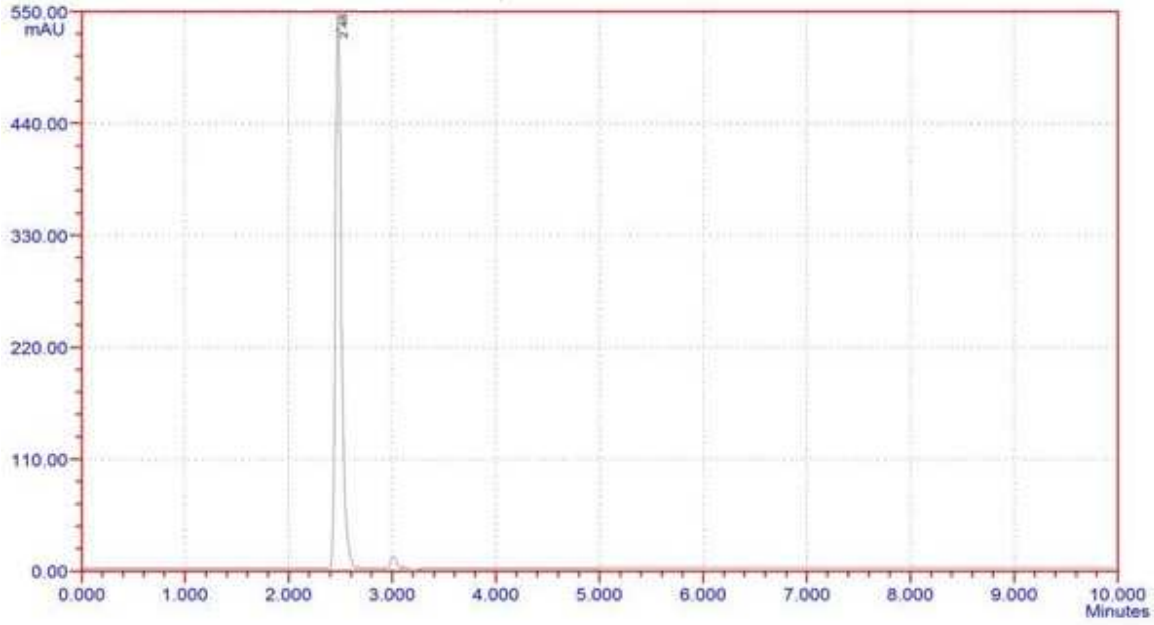
Analysis of marketed formulations

The developed method was applied to the analysis of tolperisone in pharmaceutical dosage form marketed as capsule formulation. The results of analysis are given in Table 5. The contents of the pharmaceutical dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of tolperisone in capsule dosage form as shown in figure 4.

Table 5: Assay result of capsule formulation using proposed method

Formulation	Labelled claim (mg)	% of Tolperisone in Tablet
Myolax	50	5.06

Fig. 4: RP-HPLC chromatogram of Tolperisone formulation



CONCLUSION

The above proposed study describes a new RP-HPLC method using simple mobile phase for the estimation of tolperisone in pharmaceutical dosage form. The method was validated and found to be simple, sensitive, accurate and precise. It is also proved to be convenient and effective for the determination of tolperisone in the bulk as well as pharmaceutical capsule dosage form. The percentage of recovery shows 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.

Acknowledgement
The authors are very much grateful to the management of R.V.R & J.C College of Engineering and Acharya Nagarjuna University, Guntur, India, for providing moral support in carrying out this work.

REFERENCES

[1] Porszasz J, Barankay T, Nador K, Gibiszer K, *Arzneim-Forsch Drug Res*, **1961,** 11, 257–260.
[2] Orgova´n G, Tihanyi K, Nosza´ l B, *J Pharm Biomed Anal*, **2009**, 50, 718–723.
[3] Sae-Lee N, Sae-Lee N, *SWU J Pharm Sci*, **2005,** 10, 121–124.
[4] Sae-Lee N, Sae-Lee N, *Thai Pharm Health Sci J*, **2006**, 11, 1–4.

[5] Jung-Woo Bae, Young-Seo Park, Uy-Dong Sohn, Chang-Sun Myung, Byung-Kwon Ryu, Choon-Gon Jang, and Seok-Yong Lee, *Arch Pharm Res*, **2006,** 29, 4, 339-342.
[6] Osama H Abdelmageed, *J AQAC International*, **2007**.
[7] Liawruangrath S, Liawruangrath B, Pibool P, *J Pharm Biomed Anal*, **2001**, 26, 865–872.
[8] Liawruangrath S, Liawruangrath B, *J Pharm Biomed Anal*, **1999,** 20, 401-404.
[9] V.Jagathi .Assay of tolperisone by extractive spectrophotometry, *RJPBCS*, **2010,** 1, 3, 654.
[10] P.Sai Praveen et al, *Int. J. Res. Pharm. Sci.*, **2010,** 1, 3, 317-320.
[11] Nopparat Sae.Lee, *The Pharamaceutical and health Science Journal*, **2006,** 111, 1.
[12] Senthil Kumar K, Lakshmi Siva Subramaniam, *Indian J Pharm Sci*, **2004,** 4, 799-882.
[13] Bae JW, Park YS, Sohn UD, Myung CS, Ryu BK, Jang CG, Lee SY, *Arch Pharm Res*, **2006,** 29,339–342
[14] Teruyoshi Yokoyama et al, *Chem.Pharm.Bull*, **1992,** 40, 1, 272-274.