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Estimation of Centrally Acting Muscle Relaxant Drug Tolperisone Hydrochloride Using HPTLC Method

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Abstract A sensitive, selective and precise high performance thin layer chromatographic method has been developed and validated for the determination of tolperisone hydrochloride in bulk drug and in formulation. The method employed HPTLC aluminium plates pre-coated with silica gel 60F-254 as the stationary phase while the solvent system was methanol: ethyl acetate (3:7, v/v). The R_f value of tolperisone hydrochloride was observed to be 0.50 ± 0.008. The densitometric analysis was carried out in absorbance mode at 261nm. The linear regression analysis data for the calibration plots showed a good linear relationship for tolperisone hydrochloride over a concentration range of 50 - 800 ng/spot. The limit of detection and limit of quantification for tolperisone hydrochloride was found to be 7.57 and 10ng/spot. Tolperisone hydrochloride was subjected to acid and alkali hydrolysis, chemical oxidation, wet hydrolysis, dry heat degradation and sun light degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their R_f values. Stressed samples were assayed using developed HPTLC method. The proposed method was validated with respect to linearity, accuracy, precision and robustness. The method was successfully applied to the estimation of tolperisone in tablet dosage forms. The proposed study describes a HPTLC method for the estimation of tolperisone in bulk and their pharmaceutical dosage form. Statistical analysis showed that the method is suitable for the routine analysis of tolperisone in tablets.

Keywords: *Tolperisone hydrochloride; forced degradation; HPTLC; validation*

1. Introduction

Tolperisone (TOL) 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 (Figure 1). 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. Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of tolperisone which includes potentiometry [2], spectroscopy [3,4,5], NMR analysis for kinetic study of tolperisone[6], effect of temperature and humidity on stability of tolperisone [7,8], high performance thin layer chromatography (HPTLC) [9,10,11] and high performance liquid chromatography [12,13]. 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 [14,15]. Literature review revealed that stability indicating RP-HPLC method [16] has been reported for the estimation of tolperisone in pharmaceutical dosage form. A major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces the time and cost of analysis, minimizes exposure risks, and significantly reduces disposal problems of toxic organic solvents, thereby reducing the possibilities of environment pollutionIn the proposed study, attempt has been made to develop sensitive stability indicating HPTLC method for the estimation of TOL in bulk and pharmaceutical dosage form. The proposed method was validated according to ICH guidelines [17] and its updated international convention.

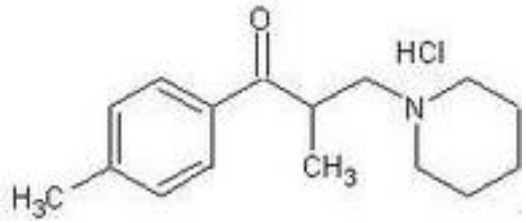

Figure 1. Chemical structure of Tolperisone hydrochloride

2. Experimental

2.1. HPTLC Instrument

The samples were applied in the form of a bands of width 6 mm with a Camag 10µl sample syringe (Hamilton, Switzerland) using Camag Linomat 5 (Switzerland) sample applicator on pre-coated silica gel aluminum plate 60 F254, (10cm x 10cm with 0.2mm thickness, E. Merck, Germany). Camag TLC scanner was used for the densitometric scanning of the developed chromatogram. All the drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

2.2. Chemicals and Reagents

Analytically pure TOL was procured as gratis samples from Sun Pharmaceutical Pvt. Ltd., Baroda, India. HPLC grade methanol and ethyl acetate were obtained from E. Merck Ltd., Mumbai, India. Tablet formulation A (Synaptol - 50 (50mg), Sun Pharmaceutical Pvt. Ltd., Silvassa, Gujarat, India) containing labelled amount of 50mg of tolperisone were purchased from local market.

2.3. Chromatographic System

2.3.1. Sample Application

Standards and formulation samples of TOL was applied on the HPTLC plates in the form of narrow bands of 6mm length, 10mm from the bottom and left edge, and with 9mm distance between two bands. Samples were applied under a continuous drying stream of nitrogen gas.

2.3.2. Mobile Phase and Development

Plates were developed using a mobile phase consisting of methanol: ethyl acetate (3:7, v/v). Linear ascending development was carried out in a twin-trough glass chamber equilibrated with the mobile phase vapors for 30min at 25℃ ± 2℃. Ten milliliters of the mobile phase (5mL in the trough containing the plate and 5mL in the other trough) was used for each development and was allowed to migrate a distance of 80mm. After development, the HPTLC plates were dried completely.

2.3.3. Densitometric Analysis

Densitometric scanning was performed in the absorbance mode under control by winCATS planar chromatography software. The source of radiation was the deuterium lamp, and bands were scanned at 261nm. The slit dimensions were 5mm length and 0.45mm width, with a scanning rate of 20mm/s. Concentrations of the compound chromatographic were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using a linear regression equation.

2.4. Preparation of Standard Stock Solution

Stock solutions were prepared by accurately weighing 25mg of TOL, transferring into 25mL volumetric flask containing a 10 mL of methanol. The flask was swirled to dissolve the solids. Volume was made up to the mark with methanol to yield a solution containing 1000µg/mL of TOL. Aliquot from the stock solution of TOL was appropriately diluted with mobile phase to obtain working standard solution of 50µg/mL of TOL.

2.5. Validation

Validation of optimized HPTLC method was done with respect to following parameters.

2.5.1. Linearity of Calibration Curves

Linearity of the method was evaluated by constructing calibration curves at eight concentration levels over a range of 50-800ng/band for TOL. The calibration curves were developed by plotting peak area versus concentration (n=5) with the help of the winCATS software.

2.5.2. Accuracy

The accuracy of the method was determined by calculating recoveries of TOL by method of standard additions. Known amount of TOL (0, 150, 300, 450ng/spot) was added to a pre quantified sample and the amount of TOL was estimated by measuring the peak area and by fitting these values to the straight-line equation of calibration curve.

2.5.3. Precision

Precision was evaluated in terms of intraday and interday precisions. For interday study sample solution of TOL were prepared in low, medium and high concentrations (50, 400, 800ng/spot) from standard stock solution. The solutions were prepared and analysed (n=3) for three different days and results were analysed. The results are reported in terms of % RSD of peak area. Similarly for Intraday study three solutions of different concentrations were analysed three times on the same day. The peak areas obtained were used to calculate mean and RSD values.

Repeatability of measurement of peak area was determined by analyzing TOL (200ng/ spot) sample seven times without changing the position of plate.

2.5.4. Specificity

The specificity was estimated by spiking commonly used excipients (starch, talc and magnesium stearate) into a pre weighed quantity of drug. The chromatogram was taken by appropriate dilutions. Developed spot area and Rf value of TOL was determined and effect of interfering compound was investigated.

2.5.5. Sensitivity

Sensitivity of the method was determined with respect to LOD and LOQ. Noise was determined by scanning a blank band (methanol) six times. A series of concentrations of drug solutions for TOL 50-800ng/band was applied on a plate and analyzed to determine LOD and LOQ. LOD was calculated as 3 times the noise level, and LOQ was calculated as 10 times the noise level. LOD and LOQ were experimentally verified by diluting the known concentrations of TOL until the average responses was approximately 3-10 times the SD of the responses for six replicate determinations.

2.5.6. Robustness

Small changes in the chamber saturation time, and solvent migration distance were introduced, and the effects on the results were examined. Robustness of the method was determined in triplicate at a concentration level of 400ng/band for TOL and the mean and RSD of peak areas were calculated.

2.6. Analysis of Marketed Formulations

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 50mg of TOL was accurately weighed and transferred to a 10mL volumetric flask. A few mL (5mL) of methanol was added to the above flask and flask was sonicated for 5min. The solution was filtered using Whatman filter paper No. 1 in another 10mL volumetric flask and volume was diluted to the mark with the methanol. 1µl of these solutions were applied to HPTLC plates and analyzed for TOL content using the proposed method as described earlier. The possibility of interference from other components of the tablet formulation in the analysis was studied. From the developed chromatogram spot area and R_f values were determined.

2.7. Forced Degradation Study

Stress degradation study using acid and alkali hydrolysis, chemical oxidation, wet hydrolysis exposure to sun light and dry heat degradation was carried out and interference of the degradation products was investigated. TOL was weighed (10mg) and transferred to 10ml volumetric flasks and expose to different stress conditions.

2.7.1. Heat Induced Alkali Hydrolysis

To the 10 ml volumetric flask, 10mg of TOL was taken and 2ml of 0.1 N NaOH was added to perform heat induced base hydrolysis. The flask was heated at 80 ℃ for 48hrs and allowed to cool to room temperature. Solution was neutralized with 0.1 N HCl and volume was made up to the mark with methanol. 0.5ml of aliquots was taken from the above solution and diluted with mobile phase to obtain final concentration of 50µg mL^{-1} of TOL.

2.7.2. Heat Induced Acid Hydrolysis

To the 10ml volumetric flask, 10mg of TOL was taken and 2ml of 0.1 N HCl was added to perform heat induced acid hydrolysis. The flask was heated at 80 ℃ for 48hrs and allowed to cool to room temperature. Solution was neutralized with 0.1 N NaOH and volume was made up to the mark with methanol. 0.5ml of aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 50µg mL^{-1} of TOL.

2.7.3. Heat Induced Wet Hydrolysis

To the 10ml volumetric flask, 10mg of TOL was taken and 2ml of HPLC grade water was added to perform heat induced wet hydrolysis. The flask was heated at 80 ℃ for 48hrs and allowed to cool to room temperature and volume was made up to the mark with methanol. 0.5ml of aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 50µg mL^{-1} of TOL.

2.7.4. Heat Induced Oxidative Stress Degradation

To heat induced perform oxidative stress degradation, 10mg of TOL was taken in 10ml volumetric flask and 2ml of 6% hydrogen peroxide was added. The mixture was heated in a water bath at 80 ℃ for 24hrs and allowed to cool to room temperature and volume was made up to the mark with methanol. 0.5ml of aliquot was taken from above solution and diluted with mobile phase to obtain final concentration of 50µg mL^{-1} of TOL.

2.7.5. Dry Heat Degradation

Analytically pure 10mg sample of TOL was exposed in oven at 80 ℃ for 48hrs. The solids were allowed to cool and transferred to volumetric flasks (10ml) and dissolved in few ml of methanol. Volume was made up to the mark with the methanol. Solution was further diluted by mobile phase to obtain final concentration of 50µg mL^{-1} of TOL.

2.7.6. Study Photolytic (Sunlight) Degradation

Analytically pure 10mg of drug were exposed to sunlight for 48hrs. The solids were allowed to cool and transferred to volumetric flask (10ml) and dissolve in few ml of methanol. Volume was made up to the mark with the methanol. Solution was further diluted with the mobile phase to obtain final concentration of 50µg mL^{-1} of TOL.

The final concentration of all the solutions 600ng/band was applied on the HPTLC plates and chromatograms were recorded.

3. Results and Discussion

3.1. Optimization of the Mobile Phase

The objective of the method development was to resolve chromatographic peaks for active drug ingredients and degradation products produced under stressed conditions with less asymmetric factor.

To develop the HPTLC method of analysis of TOL for routine analysis, selection of the mobile phase was carried out on the basis of polarity. A mobile phase that would give a dense and compact band with an appropriate Rf value for TOL was desired. Various mobile phases such as methanol-toluene, toluene-ethyl acetate, methanol-toluene-ethyl acetate, were evaluated in different proportions. A mobile consisting methanol: ethyl acetate (3:7, v/v) gave good separation of TOL from its matrix. It was also observed that chamber saturation time and solvent migration distance were crucial in the chromatographic separation as chamber saturation time of less than 30min and solvent migration distances greater than 80mm resulted in diffusion of the analyte band. Therefore, methanol: ethyl acetate (3:7, v/v) mobile phase with a chamber saturation time of 30min at 25 ℃ and solvent migration distance of 80mm was used. These chromatographic conditions produced a well-defined, compact band of TOL with optimum migration at Rf 0.50 (Figure 2). The UV spectra of the drug showed that TOL absorbed appreciably at 261nm, so detection was carried out at 261nm.

3.2. Validation

3.2.1. Linearity and Calibration Curves

Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to the concentration of the analyte. The method were found to be linear in a concentration range of 50-800ng/band (n=5) for TOL with respect to peak area. Figure 3 displays a three-dimensional overlay of HPTLC densitograms of the

calibration bands of TOL at 261nm. The regression data shown in Table 1 reveal a good linear relationship over the concentration range studied, demonstrating the suitability of the method for analysis.

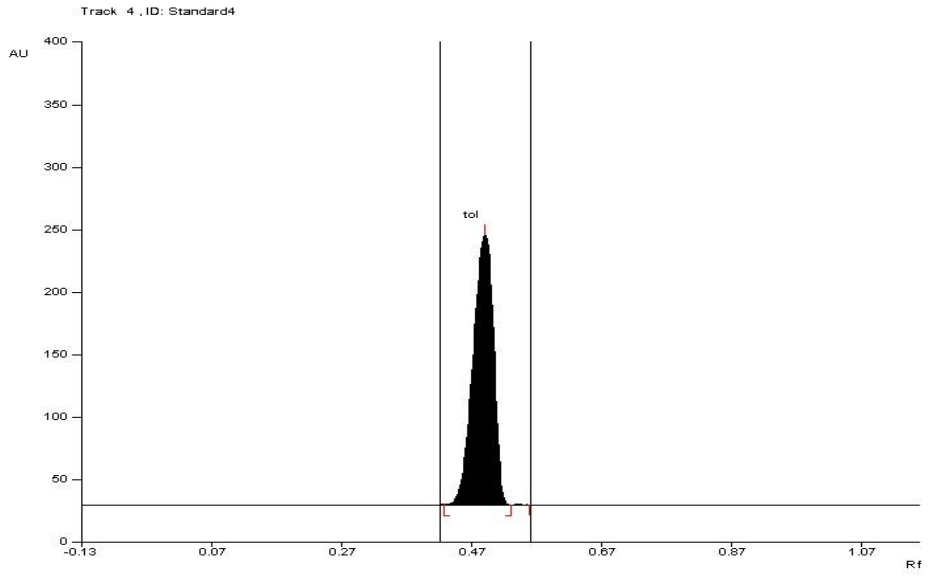

Figure 2. Densitogram of TOL using mobile phase methanol: ethyl acetate (3:7, v/v)

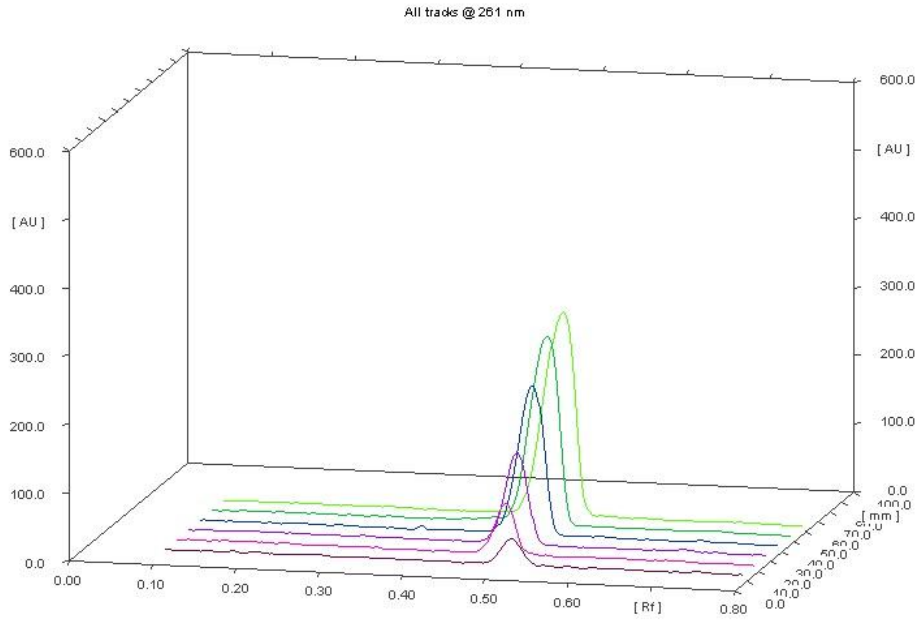

Figure 3. Three dimensional overlay of HPTLC densitograms of calibration bands of TOL

Table 1. Regression analysis of Calibration curve

Parameters	TOL
Linearity range (ng/spot)	50 -800
Slope	10.14
Standard deviation of slope	0.165
Intercept	473.94
Standard deviation of intercept	23.28
Correlation coefficient	0.9980

Table 2. Summary of validation parameters

Parameters	TOL
Rf	0.50 ± 0.008
Detection limit (ng/spot)	7.57
Quantitation limit (ng/spot)	10
Accuracy (%)	98.81 –101.11%
Precision (RSD[a], %)	
Intra-day precision (n=3)	1.88 – 2.68%
Inter-day precision (n=3)	1.93 – 3.47%
Repeatability study (%RSD) (n=7)	3.11%

[a] RSD is relative standard deviation and 'n' is number of determinations

3.2.2. Accuracy

Accuracy of an analytical method is the closeness of test results to the true value. It was determined by the application of analytical procedure to recovery studies, where a known amount of standard is spiked into pre-analyzed samples solutions. Results of the accuracy studies from excipients matrix are shown in Table 2; recovery values demonstrated the accuracy of the method in the desired range.

3.2.3. Precision

The precision of an analytical method expresses the degree of scatter among a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intraday precision refers to the use of an analytical procedure within a laboratory over

a short period of time by the same operator with the same equipment, whereas interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days. The results obtained are shown in Table 2. In all instances, RSD values were less than 5%, confirming the precision of the method. Repeatability of the scanning device was studied by applying and analyzing TOL sample (200ng/spot) seven times. RSD was less than 5% (Table 2), which was well below the instrumental specifications.

3.2.4. Limit of Detection and Limit of Quantification

Under the experimental conditions used, the lowest amount of TOL could be detected (LOD) was found to be 7.5ng/band and the lowest amount of could be quantified (LOQ) was 10ng/band. The nanogram amount of quantity of drug can be quantified which indicate that the method is sensitive.

3.2.5. Specificity

Specificity is the ability of an analytical method to determine the analyte unequivocally in the presence of sample matrix. There was no interfering peak at the Rf value of TOL from excipients added in the synthetic formulation. In addition, there was no interference from excipients present in the commercial formulation, thereby confirming the specificity of the method.

3.2.6. Robustness

The low values of RSD (Table 3) obtained after introducing small, deliberate changes in parameters of the developed HPTLC method confirmed its robustness.

Table 3. Robustness studies of TOL

Parameters	Concentration (ng/spot)	Normal condition	Change in condition	Area ± SD (n = 3)	Amount recovered (ng/spot)	% Recovery	% RSD
Chamber Saturation time	400	30 min	20 min	4541.97 ± 202.37	400.79	100.20	4.46
			40 min	4512.47 ±154.84	397.88	99.47	3.43
Mobile phase ratio Methanol : Ethyl acetate	400	4:6	3.5: 6.5	4513.2 ± 102.61	397.95	99.49	2.27
			4.5:.5.5	4530.43 ±116.92	399.65	99.91	2.58
Temperature of working area	400	25 °C	20 °C	4466.63 ± 91.16	393.36	98.34	2.04
			30 °C	4579.43 ± 149.21	404.48	101.12	3.26
Change in Wavelength	400	261	258	4522.87 ± 133.05	398.90	99.73	2.94
			264	4531 ± 178.94	399.70	99.93	3.95
Solvent for mobile phase made company	400	E. Merck	E. Merck	4506.17 ± 85.06	397.26	99.32	1.89
			SRL	4489.57 ± 123.69	395.62	98.91	2.76

3.3. Analysis of Marketed Formulation

Marketed formulation was analyzed using proposed method which gave percentage recoveries of 98.81-101.11% for TOL. A well resolved band at Rf 0.50 was observed in the chromatogram of TOL and no interference from the excipients present in the marketed tablet formulation was observed. The student's t test was applied at 95% confidence interval. The t calculated and t tabulated values were found to be 0.13 and 4.3 respectively which indicate that there is no significant difference in the assay results obtained.

3.4. Forced Degradation Study

Chromatogram of base hydrolysis performed at 80^{0}C for 48hrs reflux showed degradation of TOL with degradation product peak at retention factor (Rf) 0.35 (Figure 4). Chromatogram of acid hydrolysis performed at 80 ℃ for 48hrs reflux showed degradation of TOL with degradation product peak at retention factor (Rf) 0.69 (Figure 5).

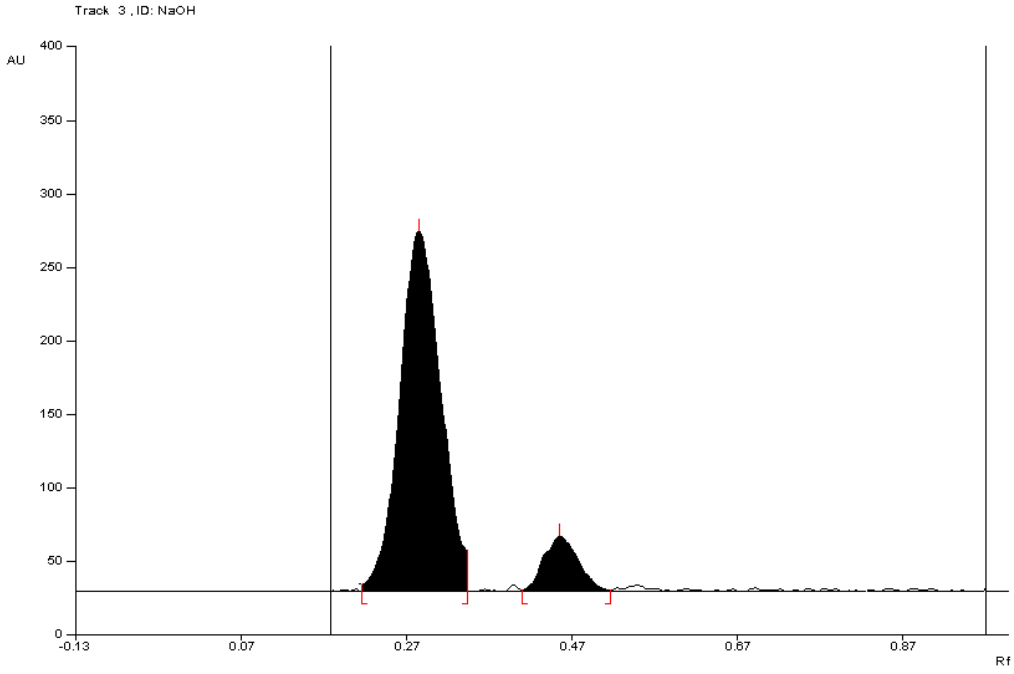

Figure 4. Densitogram of 0.1M NaOH treated TOL at 80^{0}C temperature for 48 hrs reflux

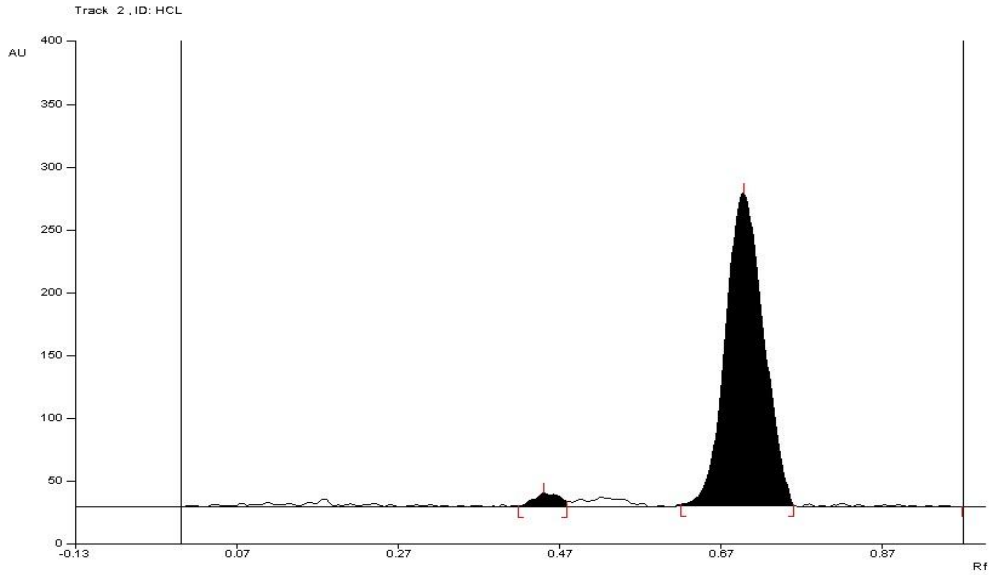

Figure 5. Densitogram of 0.1M HCl treated TOL at 80 ℃ temperature for 48hrs reflux

The chromatogram of oxidized TOL with 6% hydrogen peroxide at 80 ℃ for 24hrs reflux showed degradation of TOL with degradation product peak at retention factor (Rf) 0.62 (Figure 6). The chromatogram of TOL expose to sun light for 48hrs showed degradation of TOL with degradation product peak at retention factor (Rf) 0.70, 0.88 (Figure 7). The chromatogram of TOL exposed to dry heat at 80 ℃ for 48 hrs showed degradation of TOL with degradation product peak at retention factor (Rf) 0.70 (Figure 8).

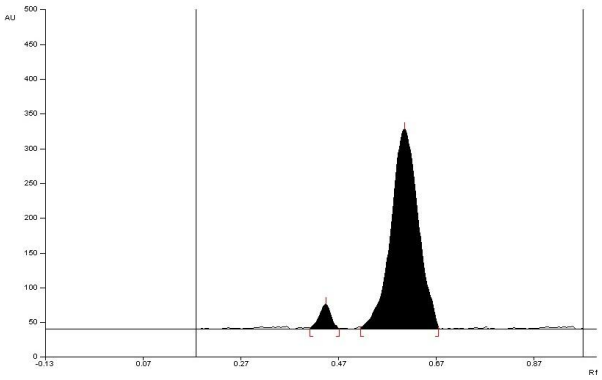

Figure 6. Densitogram of 6% H_2O_2 treated TOL at 80 ℃ temperature for 24 hrs reflux

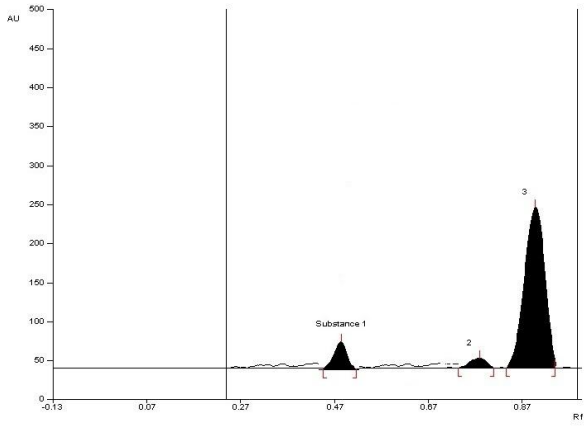

Figure 7. Densitogram of sun light treated TOL for 48hrs

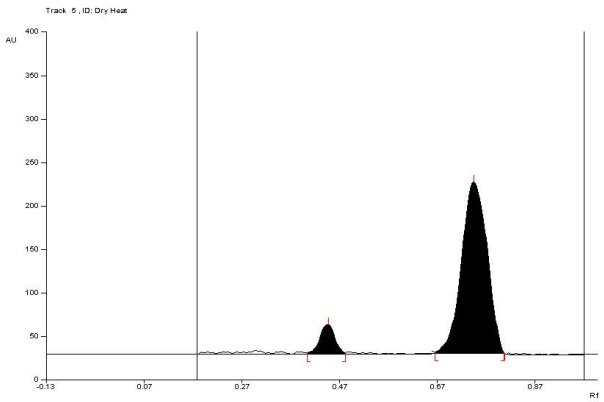

Figure 8. Densitogram of dry heat TOL at 80 ℃ temperature for 48 hrs reflux

The degradation study thereby indicated that TOL was found to be stable to wet hydrolysis while it was susceptible to base hydrolysis, acid hydrolysis, wet hydrolysis, oxidation (6% hydrogen peroxide), photo degradation, and dry heat (Table 4). The degradation peaks were well resolved from the drug peak and no degradation products from different stress conditions affected determination of TOL which indicate that the method is selective and specific.

Table 4. Forced degradation study of TOL

Conditions	Time (h)	Recovery (%)	Rf value of degradation products (Min)
Base 0.1 N NaOHa	48	10.87	0.35
Acid 0.1 N HCla	48	1.91	0.69
6% Hydrogen peroxidea	24	2.35	0.62
Dry heata	48	4.87	0.78
Light degradation	72	50.46	0.79, 0.89

[a]Samples were heated at 80°C for specified period of time

4. Conclusions

Introducing HPTLC method in pharmaceutical analysis represents a major step in quality assurance. As compared with the published method (9) the proposed method shows the degradation of tolperisone in alkali hydrolysis, acid

hydrolysis, wet hydrolysis, oxidative, dry heat degradation and photo degradation. Proposed study describes stability indicating HPTLC method for the estimation of TOL in bulk and their pharmaceutical dosage form. The method was validated and found to be sensitive, accurate and precise. Statistical analysis proved that method was repeatable and selective for the analysis of TOL without any interference from the excipients. The method was successfully used for the determination of drug in their pharmaceutical formulation. Also the above results indicated the suitability of the method for acid, base, oxidation, dry heat and photolytic degradation study. As the method separates the drugs from its degradation products, it can be used for analysis of stability samples. The method is suitable for the routine analysis of TOL in tablets. In addition, the HPTLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

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