

Research Article

Development of Stability Indicating LC Method for the Estimation of Tolperisone in Bulk and Pharmaceutical Dosage Form

U. K. Chhalotiya,¹ K. K. Bhatt,¹ D. A. Shah,¹ S. L. Baldania,¹ and S. B. Patel²

¹ Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala College of Pharmacy, Beyond GIDC Phase IV, Vithal Udyognagar, New Vallabh Vidyanagar, Anand, Gujarat 388121, India

² Department of Pharmacology, Indukaka Ipcowala College of Pharmacy, Beyond GIDC Phase IV, Vithal Udyognagar, New Vallabh Vidyanagar, Anand, Gujarat 388121, India

Correspondence should be addressed to U. K. Chhalotiya; usmangani84@gmail.com

Received 13 August 2013; Accepted 28 October 2013

Academic Editor: Rassoul Dinarvand

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A rapid, specific, and sensitive reverse phase high performance liquid chromatographic method has been developed and validated for analysis of tolperisone in both bulk and pharmaceutical dosage form. The HPLC method was performed with a reversed phase C₁₈ SunFire column (250 mm × 4.6 mm i.d., 5 mm particle size), detection at 261 nm and a mixture of methanol, water and pH 7.5 adjusted by use of 1% solution of triethylamine (60 : 40) as mobile phase. The flow rate was 1.0 mL min⁻¹ and effluents were monitored at 261 nm. The retention time of tolperisone was 4.8 min. Tolperisone was subjected to acid and alkali hydrolysis, chemical oxidation, wet hydrolysis, dry heat degradation, and sunlight degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. Stressed samples were assayed using developed LC 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.

1. Introduction

Tolperisone (TOL) a central muscle relaxant suitable for cerebral arteriosclerosis and for treating extrapyramidal 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 coordination, weakness, and mental confusion or withdrawal phenomena, in contrast to other muscle relaxants. Its molecular formula and molecular weight are C₁₆H₂₃NO·HCl and 281.83, respectively. Tolperisone HCl is extremely water soluble; it is more stable in acidic medium pH < 4.5 [2–4]. Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of tolperisone which includes potentiometry [5], spectroscopy [6–8], high performance thin layer chromatography (HPTLC) [9], and high performance liquid chromatography [10, 11]. The reported

RP-HPLC methods are aimed at quantifying tolperisone in biological fluids [12, 13]. Literature review revealed that stability indicating RP-HPLC method [14] has been reported for the estimation of tolperisone in pharmaceutical dosage form. In the proposed study, attempt has been made to develop sensitive stability indicating RP-LC method for the estimation of TOL in bulk and pharmaceutical dosage form [15].

2. Materials and Methods

2.1. Instrumentation

2.1.1. High Performance Liquid Chromatography. The liquid chromatographic system of waters (Calcutta, India) containing HPLC isocratic pump (515), variable wavelength programmable 2998 photodiode array detector, and rheodyne injector with 20 μL fixed loop was used. A SunFire C₁₈

column with 250×4.6 mm i.d. and $5 \mu\text{m}$ particle size was used as stationary phase.

2.2. Reagents and Materials. Analytically pure tolperisone (TOL) was procured from Sun Pharmaceutical Pvt. Ltd., (Baroda, India). LC grade of methanol and water was purchased from E. Merck (Mumbai, India). Triethylamine (Sisco research laboratories, Mumbai, India) was of analytical reagent grade. Tablet formulation A (Synaptol-50 (50 mg), Sun Pharmaceutical Pvt. Ltd., Silvassa, Gujarat, India) containing labelled amount of 50 mg of tolperisone was purchased from local market.

2.3. Preparation of Mobile Phase and Stock Solution. Mobile phase was prepared by mixing 950 mL of methanol with 50 mL of water. The pH was adjusted to 7.5 with 1% triethylamine. The mobile phase was filtered with Whatman filter paper no. 42 ($0.45 \mu\text{m}$). The mobile phase was sonicated for 15 min for degassing prior to use.

Stock solution was prepared by accurately weighing 10 mg of TOL and transferring to 10 mL volumetric flasks containing 3 mL of methanol. The flask was sonicated for 10 minutes to dissolve the solids. Volume was made up to the mark with methanol, which gave $1000 \mu\text{g/mL}$. 1 mL of aliquot from the stock solution was diluted with mobile phase to obtain working standards of $100 \mu\text{g/mL}$ of drug.

2.4. Chromatographic Conditions. A reversed phase C_{18} column (SunFire) equilibrated with mobile phase comprising of methanol:water (95:05 v/v; pH 7.5) was used. Mobile phase flow rate was maintained at 1 mL/min and effluents were monitored at 261 nm. A $20 \mu\text{L}$ of sample was injected using a fixed loop, and the total run time was 10 min. All the chromatographic separations were carried out at controlled room temperature ($25 \pm 2^\circ\text{C}$).

2.5. Calibration Curves for TOL. Appropriate aliquots of TOL working standard solutions were taken in different 10 mL volumetric flasks. The volume was made up to the mark with mobile phase to obtain final concentrations of 0.01, 0.1, 1, 10, 20, and $30 \mu\text{g/mL}$ of TOL, respectively. The solutions were injected using a $20 \mu\text{L}$ fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting peak area versus concentrations of the drug. The nonweighted linear regression equation was computed for TOL.

2.6. Analysis of Marketed Formulations. Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 50 mg TOL was taken in 25 mL volumetric flask. A 5 mL of methanol was added to the above flask and the flask was sonicated for 15 minutes. The solution was filtered into another 25 mL volumetric flask using Whatman filter paper no. 42 and volume was made up to the mark with the same solvent.

Appropriate aliquot was taken from above solution and transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase to obtain a solution containing $10 \mu\text{g/mL}$ of TOL. The solution was sonicated

for 10 min. It was injected as per the above chromatographic conditions and peak area was recorded. The quantifications were carried out by keeping these values to the linear equation of calibration curve.

2.7. Validation of Method. The method was validated as per ICH guideline [16] for accuracy, precision, specificity, detection limit, quantitation limit, and robustness.

2.7.1. Accuracy. Known amount of TOL (0, 5, 10, $15 \mu\text{g/mL}$) was added to a prequantified sample solutions. The amount of TOL was estimated using linear regression equation.

2.7.2. Precision. The instrument precision was evaluated by injecting the solution containing TOL ($10 \mu\text{g/mL}$) three times repeatedly and peak area was measured. The results are reported in terms of % relative standard deviation. The intraday and interday precision study of TOL was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days (first, second, and third day) for 3 different concentrations of TOL (0.01, 1, and $30 \mu\text{g/mL}$) within the calibration range and the results are reported in terms of % relative standard deviation (RSD).

2.7.3. Specificity [16]. The specificity was estimated by spiking commonly used excipients (starch, talc, and magnesium stearate) into a preweighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

2.8. Forced Degradation Study. Stress degradation study using acid and alkali hydrolysis, chemical oxidation, wet hydrolysis exposure to sunlight, and dry heat degradation was carried out and interference of the degradation products was investigated. TOL was weighed (10 mg) and transferred to 10 mL volumetric flasks and exposed to different stress conditions.

2.8.1. Heat Induced Alkali Hydrolysis. To the 10 mL volumetric flask, 10 mg of TOL was taken and 2 mL of 0.1 N NaOH was added to perform heat induced base hydrolysis. The flask was heated at 80°C for 2 hrs 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. 0.1 mL of aliquots was taken from the above solution and diluted with mobile phase to obtain final concentration of $10 \mu\text{g mL}^{-1}$ of TOL.

2.8.2. Heat Induced Acid Hydrolysis. To the 10 mL volumetric flask, 10 mg of TOL was taken and 2 mL of 0.1 N HCl was added to perform heat induced acid hydrolysis. The flask was heated at 80°C for 2 hrs 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. 0.1 mL of aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of $10 \mu\text{g mL}^{-1}$ of TOL.

2.8.3. Heat Induced Wet Hydrolysis. To the 10 mL volumetric flask, 10 mg of TOL was taken and 2 mL of HPLC grade

TABLE 1: Intraday and Interday precision study of the proposed LC method.

Conc. ($\mu\text{g/mL}$)	Intraday \pm SD ^a ($n = 3$)	% RSD	Interday \pm SD ^a ($n = 3$)	% RSD
0.01	8337.333 \pm 102.08	1.22	8268.667 \pm 104.1025	1.26
1	781779.7 \pm 7822.402	1.00	776999.7 \pm 11086.82	1.43
30	1830034 \pm 13370.88	0.73	1832868 \pm 20041.62	1.09

^aSD: standard deviation.

water was added to perform heat induced wet hydrolysis. The flask was heated at 80°C for 2 hrs and allowed to cool to room temperature and volume was made up to the mark with methanol. 0.1 mL of aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 10 $\mu\text{g mL}^{-1}$ of TOL.

2.8.4. Heat Induced Oxidative Stress Degradation. To heat induced perform oxidative stress degradation, 10 mg of TOL was taken in 10 mL volumetric flask and 2 mL of 3% hydrogen peroxide was added. The mixture was heated in a water bath at 80°C for 2 hrs and allowed to cool to room temperature and volume was made up to the mark with methanol. 0.1 mL of aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 10 $\mu\text{g mL}^{-1}$ of TOL.

2.8.5. Dry Heat Degradation. Analytically pure 10 mg sample of TOL was exposed in oven at 80°C for 2 hrs. The solid was allowed to cool and was transferred to volumetric flasks (10 mL) and dissolved in few mL of methanol. Volume was made up to the mark with the methanol. Solution was further diluted with mobile phase to obtain final concentration of 10 $\mu\text{g mL}^{-1}$ of TOL.

2.8.6. Photolytic Degradation. Analytically pure 10 mg of drug was exposed to sunlight for 24 hrs. The solid was allowed to cool and was transferred to volumetric flask (10 mL) and dissolved 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 10 $\mu\text{g mL}^{-1}$ of TOL.

All the solutions were injected in the liquid chromatographic system and chromatograms were recorded.

2.8.7. Limit of Detection and Quantification. The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines. $\text{LOD} = 3.3 \times \sigma/S$ and $\text{LOQ} = 10 \times \sigma/S$, where σ is the standard deviation of y -intercepts of regression lines and S is the slope of the calibration curve.

2.8.8. Robustness. Robustness of the method was studied by deliberately changing the experimental conditions like flow rate and percentage of organic phase and also by observing the stability of the sample solution at $25 \pm 2^\circ$ for 24 h. The sample solution was assayed at every 2 h interval up to 24 h.

3. Result

3.1. Validation of the Proposed Methods

3.1.1. Linearity. The calibration curve ($n = 5$) for TOL was found to be linear in the range of 0.01–30 $\mu\text{g mL}^{-1}$ with a correlation coefficient of 0.9990. The standard deviation value of slope and intercept of TOL was found to be 605.36 and 8210.51, respectively, which indicated strong correlation between peak area and concentration. The average ($n = 5$) regression equation of calibration curves was obtained as $y = 61442x + 11138$.

3.1.2. Precision. Instrument precision was determined by performing injection repeatability test and the % RSD value for TOL was found to be 1.02. The intraday and interday precision studies were carried out and the % RSD value was found to be 0.73–1.22 and 1.09–1.43, respectively (Table 1). The low RSD values indicate that the method is precise.

3.1.3. Accuracy. The accuracy of the method was determined by calculating recoveries of TOL by method of standard addition. The recoveries were found to be 97.85–101.21% for TOL (Table 2). The high values indicate that the method is accurate.

3.1.4. Limit of Detection and Limit of Quantification. The detection limit and quantitation limit for TOL was 0.005 $\mu\text{g mL}^{-1}$ and 0.01 $\mu\text{g mL}^{-1}$, respectively. The above data shows that a nanogram quantity of the drug can be accurately and precisely determined.

3.1.5. Specificity. The specificity study was carried out to check the interference from the excipients used in the formulation by preparing synthetic mixture containing the drug and excipients. The chromatogram showed peaks for the drug without any interfering peak.

3.1.6. Robustness. The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in Table 4. The low value of relative standard deviation was indicating that the method was robust.

3.2. Forced Degradation Study. Chromatogram of base hydrolysis performed at 80°C for 2 hrs reflux showed degradation of TOL with degradation product peak at retention times (RT) 3.261, 3.91 min, and 4.02 min (Figure 3). Chromatogram of acid hydrolysis performed at 80°C for 24 hrs

TABLE 2: Accuracy study of the proposed LC method.

Amount of sample ($\mu\text{g/mL}$)	Sets	Amount drug of spiked ($\mu\text{g/mL}$)	Area ($n = 3$)	Average amount recovered ($\mu\text{g/mL}$)	% recovery	Mean % recovery	% RSD
10	1	0	610609	9.79	97.57	97.85	1.22
	2	0	605849		96.79		
	3	0	620523		99.18		
10	1	5	924702	15.01	98.69	100.11	1.01
	2	5	932138		99.89		
	3	5	943422		101.73		
10	1	10	1238944	19.96	99.83	99.63	0.51
	2	10	1243225		100.53		
	3	10	1230848		98.51		
10	1	15	1534587	25.12	97.95	101.21	1.15
	2	15	1560664		102.19		
	3	15	1568607		103.49		

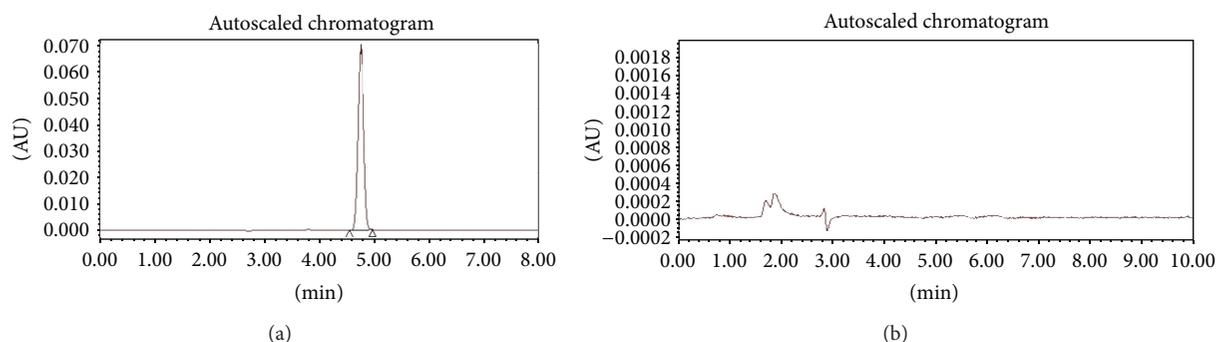
FIGURE 1: (a) Chromatogram of TOL (RT 4.8) on C_{18} SunFire column using methanol : water (95 : 05, v/v, pH 7.5 adjusted with 1% solution of triethylamine) as the mobile phase. (b) Chromatogram of placebo.

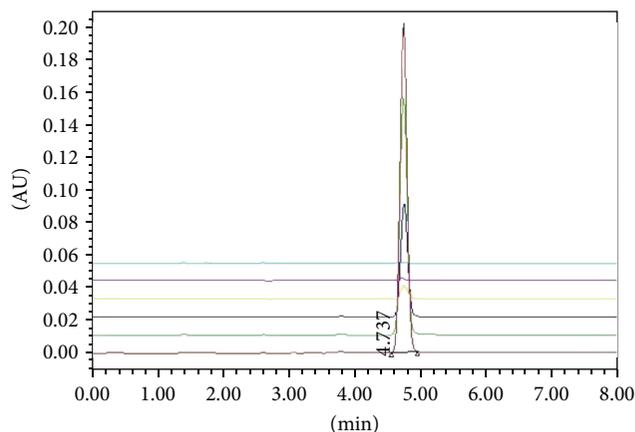
TABLE 3: Data derived from forced degradation study of TOL for the proposed LC method.

Condition	Time	% recovery TOL	Retention time of degradation products (minutes)
0.1 N NaOH ^a	2 hrs	66.69	3.26, 3.91, 4.02
0.1 N HCl ^a	24 hrs	85.70	4.03
3% H ₂ O ₂ ^a	2 hrs	86.30	4.02
Dry heat ^a	2 hrs	83.50	6.78
Sunlight	24 hrs	82.58	4.10, 4.63, 5.96

^aSamples were heated at 80°C for specified period of time.

reflux showed degradation of TOL with degradation product peak at retention time (RT) 4.03 min (Figure 4).

The chromatogram of oxidized TOL with 3% hydrogen peroxide at 80°C for 2 hrs reflux showed degradation of TOL with degradation product peak at retention time (RT) 4.02 min (Figure 5). The chromatogram of TOL exposed to dry heat at 80°C for 2 hrs showed degradation of TOL with degradation product peak at retention time (RT) 6.76 min (Figure 6). The chromatogram of TOL exposed to sunlight for

FIGURE 2: Overlay chromatogram of TOL (RT 4.737) on C_{18} SunFire column using methanol : water (95 : 05, v/v, pH 7.5 adjusted with 1% solution of triethylamine) as the mobile phase.

24 hrs showed degradation of TOL with degradation product peak at retention times (RT) 3.63, 4.10 min, and 5.96 min (Figure 7).

TABLE 4: Data derived from robustness of TOL for the proposed LC Method.

Parameters	Normal condition	Change in condition	Area ($n = 3$)	% recovery	Change in % RSD
Flow rate	1.0 mL/min	0.9 mL/min	75773.67	100.36	1.21
		1.1 mL/min	73762.33	101.92	1.02
Mobile phase ratio	Methanol : water (95 : 05)	(97 : 03)	71898.33	98.83	1.15
		(93 : 07)	71966.33	99.00	0.92
pH of mobile phase	pH 7.5	7.0	73415.33	101.36	1.08
		8.0	72573	99.99	0.97

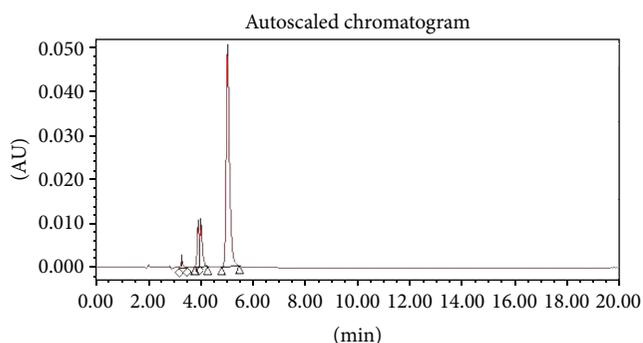


FIGURE 3: Chromatogram of 0.1 M NaOH treated TOL at 80°C temperature for 2 hrs reflux.

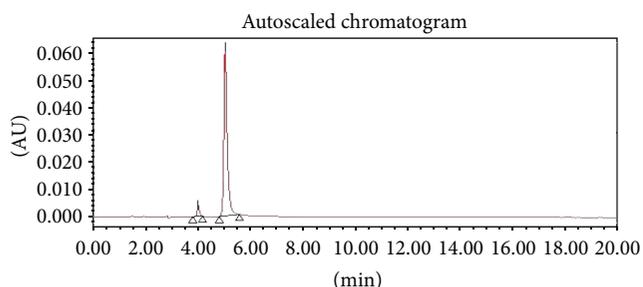
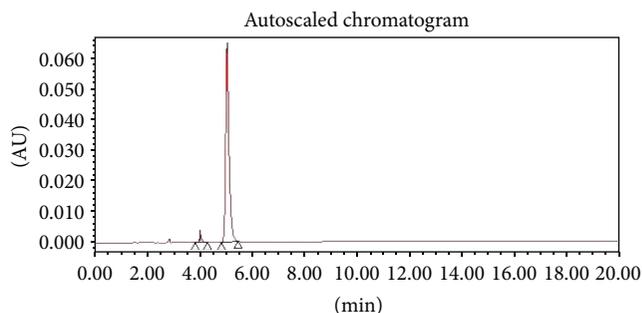


FIGURE 4: Chromatogram of 0.1 M HCl (acid hydrolysis) treated TOL at 80°C temperature for 24 hrs reflux.

The degradation study thereby indicated that TOL was found to be susceptible to base hydrolysis, acid hydrolysis, oxidation (3% hydrogen peroxide), photo degradation, and dry heat (Table 3). No degradation products from different stress conditions affected the determination of TOL.

3.3. Solution Stability. The solution stability study showed that TOL was evaluated at room temperature for 24 hr. The relative standard deviation was found below 2.0%. It showed that solution was stable up to 24 hrs at room temperature.

3.4. Analysis of Marketed Formulations. The proposed method was successfully applied to the determination of TOL in their tablet dosage form (Tablet A). The % recovery for TOL for Tablet "A" was found to be 99.21 ± 1.28 mean value standard deviation of three determinations which was comparable with the corresponding labelled amounts.

FIGURE 5: Chromatogram of 3% H₂O₂ treated TOL at 80°C temperature for 2 hrs reflux.

4. Discussion

4.1. Optimization of 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.

Various mixtures containing water, methanol, and acetonitrile were tried as mobile phases in the initial stage of method development. Mixture of methanol : water (90 : 10, v/v), methanol-water (60 : 40, v/v), and acetonitrile-water (50 : 50, v/v) were tried as mobile phase but satisfactory resolution of drug and degradation peaks were not achieved.

The mobile phase methanol : water (95 : 05, v/v pH adjusted with 1% solution of TEA) was found to be satisfactory and gave symmetric peak for TOL. The retention time for the proposed method was found to be 4.8 min as shown in Figure 1(a). The chromatogram of placebo is shown in Figure 1(b) and the overlay chromatograms of calibration range were shown in Figure 2. The system suitability parameters, theoretical plates per meter and asymmetric factor for TOL, were found to be 3805 and 0.87, respectively. The mobile phase flow rate was maintained at 1.0 mL min⁻¹. The UV spectra of the drug showed that TOL was absorbed appreciably at 261 nm, so detection was carried out at 261 nm.

4.2. Method Validation. The calibration curve was found to be linear over the range of 0.01–30 µg/mL for TOL. Instrument precision was determined by performing injection repeatability test and the % RSD value for TOL was found to be 0.74–1.24%. The intraday and interday precision studies were carried out. For the intraday study % RSD values were found to be 0.73–1.22% for TOL and for interday precision

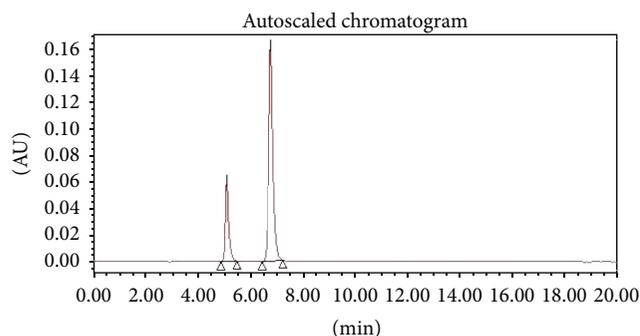


FIGURE 6: Chromatogram of dry heat TOL at 80°C temperature for 2 hrs reflux.

study % RSD values were found to be 1.09–1.43% for TOL. The low % RSD values indicate that the method is precise.

The accuracy of the method was determined by calculating recoveries of TOL by method of standard additions. The recovery obtained was 98.61–101.21% for TOL. The values indicate that the method is accurate.

The detection limits for TOL were 0.005 $\mu\text{g}/\text{mL}$ while quantitation limits were 0.01 $\mu\text{g}/\text{mL}$. The above data shows that a nanogram quantity of the drug can be accurately and precisely determined. System suitability test was carried out on freshly prepared standard stock solution of TOL.

The liquid chromatogram of the placebo used in the specificity study did not give any interfering peak in the chromatogram, which suggests that the proposed LC method is both selective and specific.

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. The low value of percentage relative standard deviation was indicating that the method was robust.

The solution stability study revealed that TOL solution was stable for 24 h without detection of degradation. The percentage recoveries of both the drugs were found to be satisfactory.

4.3. Forced Degradation Study. The degradation study thereby indicated that TOL was found to be susceptible to base hydrolysis, acid hydrolysis, oxidation (3% hydrogen peroxide), dry heat and photo degradation. No degradation products from different stress conditions affected the determination of TOL.

5. Conclusion

As compared with the published, the proposed method is more sensitive. The published method [15] shows the degradation of tolperisone in oxidative stress condition while the proposed method shows the degradation of tolperisone in alkali hydrolysis, wet hydrolysis, oxidative, dry heat degradation, and photo degradation. There was no degradation reported in acid and base hydrolysis in published paper which might be because of less severe conditions (0.01 N HCl, 0.01 N NaOH) used to perform stress testing.

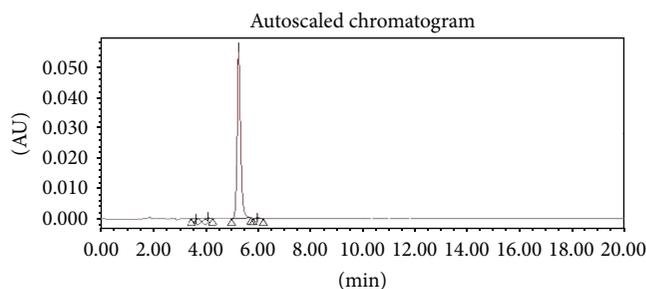


FIGURE 7: Chromatogram of sunlight treated TOL for 24 hrs.

The proposed study describes stability indicating LC method for the estimation of TOL in bulk and their pharmaceutical dosage form. The method was validated and found to be simple, sensitive, accurate, and precise. Statistical analysis proved that method to be 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 HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

Acknowledgments

The authors are thankful to Sun Pharmaceuticals Ltd., Baroda, for providing gratis sample of asenapine. The authors are very thankful to SICART and Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Anand, for providing the necessary facilities to carry out research work.

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