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Research Article

A Stability Indicating UPLC Method for the Determination of Tramadol Hydrochloride: Application to Pharmaceutical Analysis

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The use of Ultra Performance Liquid Chromatography (UPLC), with a rapid 5-minute reversed phase isocratic separation on a 1.7 μm reversed-phase packing material to provide rapid "high throughput" support for tramadol hydrochloride (TMH) is demonstrated. A simple, precise and accurate stability-indicating isocratic UPLC method was developed for the determination of TMH in bulk drug and in its tablets. The method was developed using Waters Aquity BEH C18 column (100 mm × 2.1 mm, 1.7 μm) with mobile phase consisting of a mixture of potassium dihydrogen phosphate buffer of pH 2.8 and an equal volume of acetonitrile (60 : 40 v/v). The eluted compound was detected at 226 nm with a UV detector. The standard curve of mean peak area versus concentration showed an excellent linearity over a concentration range 0.5–300 μg mL^{-1} TMH with regression coefficient (r) value of 0.9999. The limit of detection (S/N = 3) was 0.08 μg mL^{-1} and the limit of quantification (S/N = 10) was 0.2 μg mL^{-1}. Forced degradation of the bulk sample was conducted an accordance with the ICH guidelines. Acidic, basic, hydrolytic, oxidative, thermal and photolytic degradation were used to assess the stability indicating power of the method. TMH was found to degrade significantly in acidic, basic and oxidative stress conditions and stable in thermal, hydrolytic and photolytic conditions.

1. Introduction

Tramadol hydrochloride (TMH), chemically known as (1R,2R)-rel-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol (Figure 1), is a synthetic analogue of codeine and is a centrally acting analgesic agent [1]. It is metabolized by the cytochrome P450 enzyme system in the liver to form eleven metabolites of which o-desmethyltramadol (M1) predominates and has analgesic properties [2]. It has been used since 1977 for the relief of severe physical pain and has been the most widely sold opioid analgesic drug in the world [3]. TMH is official in European Pharmacopeia (EP) [4] which describe nonaqueous titration with perchloric acid as titrant the end point being located potentiometrically. Ultraviolet spectrophotometry [5, 6], high-performance liquid chromatography [6–9], thin layer chromatography-densitometry [10], capillary isotachophoresis [11], flow injection chemiluminescence spectrometry [12], voltametry [13–15], ion-selective-based potentiometry [16–22], visible spectrophotometry [23–28], and titrimetry [26, 29] for determining TMH in pharmaceutical dosage forms.

In addition, there have been reports of its assay when present in combination with other drugs. TDH and ibuprofen were assayed simultaneously by first-order derivative spectrophotometry and HPLC with UV-detection [30]. TMH along with dexketoprofen and haloperidol have been determined by HPLC-diode array detection (DAD) method [31]. Simultaneous analysis of TMH and aceclofenac in a commercial tablet was accomplished also by HPLC with UV-detection method [32]. Simultaneous determination of paracetamol, TMH, and domperidone in combined dosage

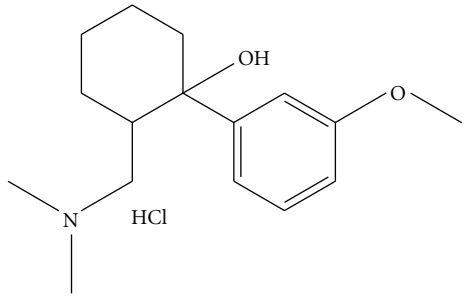

Figure 1

Table 1: Linearity and regression parameters with precision data.

Parameter	Value
Linear range, μg mL^{-1}	0.5–300
Limits of quantification, (LOQ), μg mL^{-1}	0.2
Limits of detection, (LOD), μg mL^{-1}	0.08
Regression equation	
Slope (b)	30620.1
Intercept (a)	19330.0
Correlation coefficient (r)	0.9999
Standard deviation of b, (S_b)	76.0
Standard deviation of a, (S_a)	13694.5

form using RP-HPLC has been reported by Karunakaran et al. [33].

In recent years, there has been an increased tendency towards development of stability-indicating analysis [34–37], using the approach to stress testing enshrined in International Conference on Harmonisation (ICH) guideline Q2A(R2) [38]. This approach is being extended to pharmaceuticals to enable accurate and precise quantification of drugs in the presence of their degradation products. Though there are many reported methods for the analysis of TMH either alone [5–29] or in combination with other drugs [30–32] in pharmaceutical dosage forms, the literature on the stability-indicating methods is scarce. Mohammadi et al. [39] have evaluated the stability of tramadol enantiomers using a chiral stability-indicating capillary electrophoresis method and its application to pharmaceutical analysis. Chemical stability of TMH in injection has been reported by Gupta [40].

Ultraperformance liquid chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption [41]. UPLC system is designed in a special way to withstand high system backpressure. Special analytical columns UPLC Acquity UPLC BEH C_{18} packed with 1.7 μm particles are used in the system. The UPLC system allows shortening analysis time upto nine times compared to the conventional HPLC system, but separation efficiency remains the same or is even improved. As efficiency and speed of analysis are of great importance in many applications of liquid chromatography, especially in pharmaceutical, toxicological, and chemical analysis, where it is important to increase throughput and reduce analysis costs, UPLC could play a significant role in the future of liquid chromatography.

Three reports are found in the literature dealing with the application of UPLC with mass spectrometric detection for the determination of TMH in different matrices. A sensitive UPLC-MS/MS method for TMH in urine and whole blood in forensic context has very recently been reported [42]. Kasprzyk-Hordern et al. [43] determined TMH in surface water by solid-phase extraction and UPLC-positive electrospray ionization tandem mass spectrometry. Simultaneous screening and quantification of 29 drugs of abuse including TMH in oral fluid [44] has been reported by Badawi et al. Solid phase extraction and UPLC-MS/MS by Liquid chromatography-positive electrospray ionization tandem mass spectrometry has recently [45] been used for the multiresidue analysis of drugs of abuse in waste water and surface water.

Though a number of liquid chromatographic methods have earlier proposed for TMH [5–8, 29–31] no attempt has been made to apply this new type of LC (UPLC) for pharmaceuticals except in body fluids, oral fluids, and surface and waste waters [42–45] despite its many fold advantages.

The aim of this work was to develop a rapid, precise, accurate, and validated stability-indicating UPLC method for the determination of tramadol HCl in bulk and tablets. This was accomplished with a Waters Acquity UPLC system and Acquity BEH column (C18, 100 mm, 2.1 mm, and 1.7 μm). The stability indicating power of the method was established by comparing the chromatograms obtained under optimized conditions before forced degradation with those after degradation via acidic, basic, hydrolytic, oxidative, thermal, and photolytic stress conditions.

2. Experimental

2.1. Materials and Reagents. Pharmaceutical grade TMH was kindly supplied by Jubilant Life Sciences Limited, Mysore, India, as gift and used as received. Commercial dosage forms used: Nobligan 50 and Meridol 50 tablets were purchased from local commercial sources. HPLC grade acetonitrile was purchased from Merck India. Pvt. Ltd., Mumbai, India, doubly distilled water was used throughout the investigation.

2.2. Chromatographic Conditions and Equipments. Chromatography was performed on a Waters Acquity UPLC system (Waters, Manchester, UK) using an Acquity BEH column (C-18 100 mm, 2.1 mm, and 1.7 μm; Waters, Manchester, UK) equipped with binary solvent delivery pump, autosampler and tunable UV (TUV) detector. The column oven temperature was maintained at 35°C and the autosampler temperature maintained at ambient. Isocratic mobile phase flow was carried out throughout the run. Total cycle time was 5 min with a flow rate of 0.5 mL^{-1} min and an injection volume of 4 μL using partial loop mode. The output signal was monitored and processed using Empower-2 software.

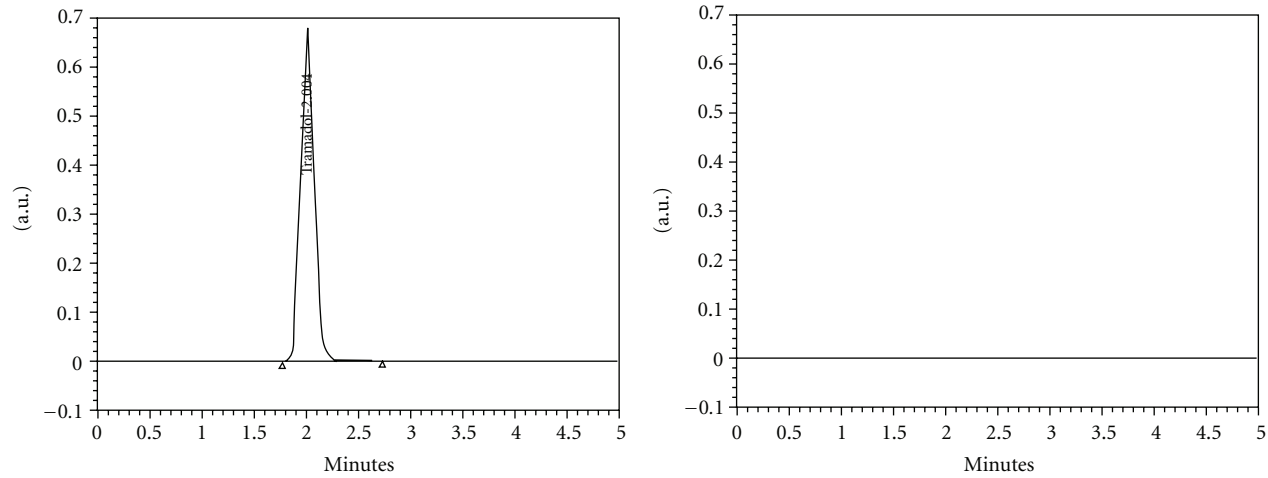

Figure 2: (a) Typical chromatogram of TMH (200 μg mL^{-1}), (b) (Blank).

Table 2: Results of accuracy study ($n = 5$).

Concentration of TMH injected, μg mL^{-1}	Intraday		Interday	
	Concentration of TMH found, μg mL^{-1}	RE, %	Concentration of TMH found, μg mL^{-1}	RE, %
150	150.80	0.54	151.03	0.69
200	199.48	0.26	198.24	0.88
250	249.16	0.34	252.12	0.85

Table 3: Results of precision study ($n = 5$).

Concentration injected, μg mL^{-1}	Intraday precision			Interday precision		
	Mean area ± SD	%RSD[a]	%RSD[b]	Mean area ± SD	%RSD[a]	%RSD[b]
150	4640550 ± 9012	0.19	0.28	4621200 ± 18068	0.39	0.23
200	6136915 ±10694	0.17	0.13	6124962 ±49690	0.81	0.19
250	7657557± 20715	0.35	0.27	7719500 ± 40184	0.52	0.78

[a]Relative standard deviation based on peak area.
[b]Relative standard deviation based on retention time.

Table 4: Method robustness.

Condition	Modification	Mean Peak area ± SD*	%RSD	Mean Rt ± SD*	%RSD	Mean theoretical plates ± SD*	%RSD	Mean tailing factor ± SD*	%RSD
Temperature	35°C	6207527 ± 6506	0.10	2.015 ± 0.006	0.30	3467 ± 59.65	72	1.10 ± 0.02	0.18
	40°C	6136916 ± 10695	0.17	2.007 ± 0.007	0.33	3953 ± 85.36	16	1.01 ± 0.01	0.10
	45°C	6186601 ± 6723	0.11	1.99 ± 0.006	0.30	3998 ± 56.56	41	1.11 ± 0.02	0.18
Mobile phase composition	40 : 60	6134526 ± 10115	0.16	1.9 ± 0.006	0.30	4021 ± 10536	62	1.02 ± 0.05	0.49
	50 : 50	6188507 ± 9437	0.15	2.0 ± 0.006	0.30	4068 ± 70.56	73	1.01 ± 0.04	0.40
	60 : 40	6345681 ± 9986	0.16	1.99 ± 0.08	0.40	4098 ± 86.97	12	0.98 ± 0.05	0.51
Flow rate	0.35	7107242 ± 8520	0.12	2.01 ± 0.05	0.25	3987 ± 101.16	53	1.02 ± 0.02	0.20
	0.40	6112464 ± 8817	0.14	2.006 ± 004	0.20	4016 ± 88.84	19	1.02 ± 0.03	0.29
	0.45	5455471 ± 9053	0.17	1.99 ± 0.05	0.25	3987 ± 101.16	53	1.02 ± 0.02	0.20
Wavelength	224 nm	7024544 ± 15608	0.22	2.00 ± 0.06	0.30	3136 ± 95.16	03	1.01 ± 0.03	0.30
	226 nm	6128799 ± 10636	0.18	2.01 ± 0.08	0.40	4268 ± 101.3	10	1.01 ± 0.04	0.40
	228 nm	7131156 ± 12845	0.18	2.00 ± 0.004	0.20	3334 ± 88.58	66	1.02 ± 0.08	0.78

*Mean value of three determinations.

2.3. Mobile Phase Preparation. Dissolved 0.5 g of potassium dihydrogen orthophosphate in 1000 mL of water and the pH was adjusted to 2.8 using 10% acetic acid. A 600 mL portion of this resulting buffer was mixed with 400 mL of acetonitrile, shaken well, and filtered using 0.22 μm Nylon membrane filter. This solution was also used as diluent in all subsequent preparations of the sample.

2.4. Instrumental Parameters. The isocratic flow rate of mobile phase was maintained at 0.5 mL min^{-1}. The column temperature was adjusted to 35°C. The injection volume was 4.0 μL. The sample run was monitored at 226 nm and the run time was 5.0 min. The retention time of the sample was observed at about 2.0 min.

2.5. Stress Study. All stress decomposition studies were performed at an initial drug concentration of 200 μg mL^{-1} in mobile phase. Acid hydrolysis was performed in 1 M HCl at 80°C for 2 h. The study in alkaline condition was carried out in 1 M NaOH at 80°C for 2 h. For study in neutral condition, drug dissolved in water was heated at 80°C for 2 h. Oxidative studies were carried out at 80°C in 5% hydrogen peroxide for 2 h. For photolytic degradation studies, pure drug in solid state was exposed to 1.2 million lux hours in a photo stability chamber. Additionally, the drug powder was exposed to dry heat at 105°C for 3 h. Samples were withdrawn at appropriate time, cooled, and neutralized by adding base or acid and subjected to UPLC analysis after suitable dilution.

2.6. Preparation of Stock Solution. Accurately weighed 100 mg of pure TMH was dissolved in and diluted to mark in a 100 mL standard flask with the mobile phase.

2.7. Procedures

2.7.1. Procedure for Preparation of Calibration Curve. Working standard solutions containing 0.5–300 μg mL^{-1} TMH were prepared by serial dilutions of aliquots of the stock solution. Aliquots of 4 μL were injected (six injections) and eluted with the mobile phase under the reported chromatographic conditions. The average peak area versus the concentration of TMH in μg mL^{-1} was plotted. Alternatively, the regression equation was derived using mean peak area-concentration data and the concentration of the unknown was computed from the regression equation.

2.7.2. Preparation of Tablet Extracts and Assay Procedure. Twenty Nobligan tablets (each containing 50 mg TMH) were accurately weighed and ground into a fine powder. Powder equivalent to 20 mg TMH was transferred into a 100 mL volumetric flask and 60 mL of the mobile phase was added. The mixture was sonicated for 20 min to achieve complete dissolution of TMH, and the content was then diluted to volume with the mobile phase to yield a concentration of 200 μg mL^{-1} TMH, and filtered through a 0.22 μm nylon membrane filter. The tablet extract was injected to the UPLC column. The same procedure was repeated with Meridol 50 (each tablet contained 50 mg TMH) tablets.

2.8. Procedure for Method Validation

2.8.1. Accuracy and Precision. Six injections, of three different concentrations (150, 200, and 250 μg mL^{-1}), were given on the same day and the values of relative standard deviation (RSD) were calculated to determine intraday precision. These studies were also repeated on different days to determine interday precision.

2.8.2. Limits of Detection (LOD) and Quantification (LOQ). Signal-to-noise (S/N) ratio method was adopted to obtain the limit of quantification (LOQ) and limit of detection (LOD). Series of dilutions of the TMH stock solution was made to attain LOQ and LOD in acceptable values. LOQ solution was injected six times ($n = 6$) and calculated the %RSD values for the obtained TMH peak area and retention time.

2.8.3. Linearity. Seven-point calibration curves were obtained in a concentration range from 0.5 to 300 μg mL^{-1} (0.5, 50, 100, 150, 200, 250, and 300 μg mL^{-1} levels) for TMH; three independent determinations were performed at each concentration.

2.8.4. Robustness and Ruggedness. To determine the robustness of the method the experimental conditions were deliberately changed. The flow rate of the mobile phase (0.5 ± 0.05 mL min^{-1}), column oven temperature (35 ± 5°C), mobile phase composition (66 : 40, 54 : 40, 60 : 36 and 60 : 44; buffer : solvent mixture v/v), and detection wavelength (226 ± 1 nm) were the varied parameters. In each case, the %RSD values were calculated for the obtained peak area and retention time. The number of theoretical plates and tailing factors were compared with those obtained under the optimized conditions. Three different columns of same dimensions were used for the analyses. The study was performed in a same day and three different days by three different analysts for three different concentrations of TMH (triplicate injections). The area obtained from each concentration was compared with that obtained under optimized conditions. The relative standard deviation values were evaluated for each concentration.

2.8.5. Solution Stability and Mobile Phase Stability. Stability of TMH solution was studied by injecting the sample into the chromatographic system at different time interval. The peak area was recorded in the time intervals of 0, 12, and 24 hrs and the RSD values were calculated. Freshly prepared solution was injected at the same time intervals for mobile phase stability (0, 12, and 24 hours) and RSD values of the peak areas were calculated.

3. Results and Discussion

3.1. Method Development. Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, col-

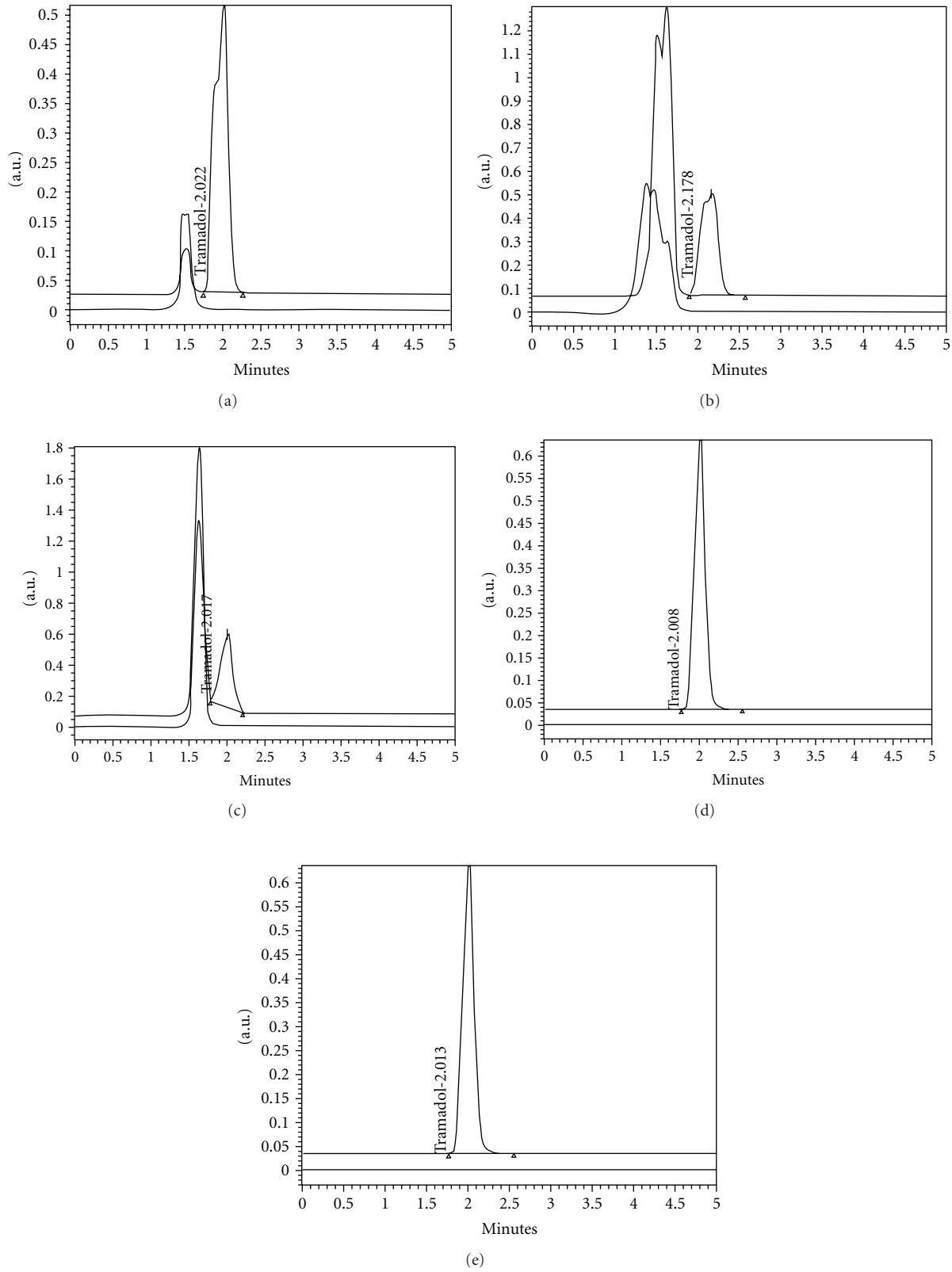

Figure 3: (a) Acid degradation, (b) base degradation, (c) oxidative degradation, (d) thermal degradation, and (e) photo degradation.

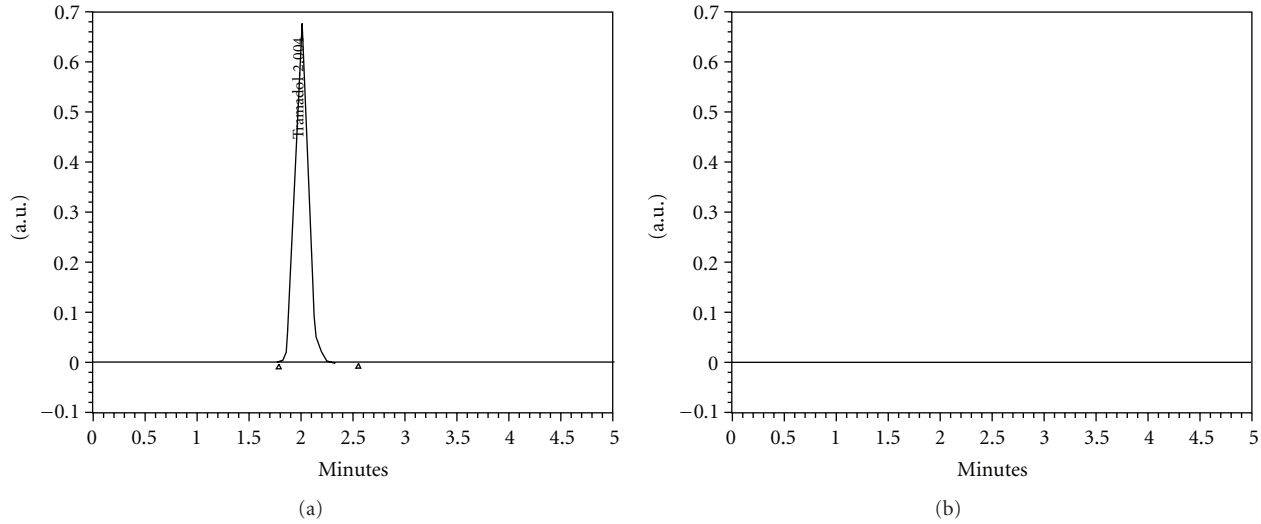

Figure 4: (a) Chromatogram of tablet extract, (b) chromatogram of placebo blank.

Table 5: Method ruggedness.

Variable	Mean Peak area ± SD*	%RSD	Mean Rt ± SD*	%RSD	Mean theoretical plates ± SD*	%RSD	Mean tailing factor ± SD*	%RSD
Analyte ($n = 3$)	6117724 ± 12676	0.21	2.005 ± 0.03	0.15	4076 ± 9634	2.36	1.03 ± 0.003	0.29
Column ($n = 3$)	6123741 ± 11896	0.19	2.007 ± 0.04	0.20	4167 ± 1034	2.48	1.02 ± 0.002	0.20

* Mean value of three determinations

umn temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Acquity BEH C18, 50 mm × 2.1 mm, 1.7 μm column used for the elution, but the peak eluted before 1.5 minutes with a tailing factor of 2. Experiment with Phenyl 100 mm × 2.1 mm, 2 μm column ended with inconsistent retention time and peak fronting. Buffers like sodium dihydrogenothophosphate, dipotassium hydrogenorthophosphate, and disodium hydrogenorthophosphate did not yield desired results. Use of ion pair reagents also did not yield the expected peak. The following gradient conditions were experimented; the cycle time was set at 5 min, 10 min, 15 min, or 20 min, while the flow rate was set at either 300 μL min^{-1} or 600 μL min^{-1}. Except for the 5 min cycle time, all gradients began with 100% buffer for 0.5 min and maintained for 1 min at the end of each cycle for equilibration. For a cycle time of 5 min, conditions started with 100% buffer for 0.5 min, then proceeded with a linear gradient to 100% acetonitrile for 3 min, then returned to initial conditions and maintained upto 5 min.The gradient method was successful in good peak shape of drug and all chromophoric degradation products. The effect of different elution gradients was assessed under either linear (described above) curve or step gradient which was controlled by the Waters Empower-2 software. At 60 : 40 ratio of the mobile phase in the linear gradient program, a perfect peak was eluted. Thus the mobile phase ratio was fixed at 60 : 40 (buffer : solvent) in an isocratic mobile phase flow rate. The typical chromatograms obtained for blank and pure TMH from final UPLC conditions are depicted in Figure 2.

3.2. Stability Studies. All forced degradation studies were analyzed at 200 μg mL^{-1} concentration level. The observation was made based on the peak area of the respective sample. TMH was found to be more stable under photolytic (1.2 million lux hours), thermal (105°C for 3 hours) in solid state, and hydrolytic (aqueous, 80°C for 2 hours) stress conditions. The drug was found to be sensitive to acid and alkaline stress conditions resulting in the decomposition to the extent of 44.3 and 34.6%, respectively. Less degradation occurred under oxidative stress conditions with percent decomposition being only 2.1%. The chromatograms obtained for TMH after subjecting to degradation are presented in Figure 3. Assay study was carried out by the comparison with the peak area of TMH sample without degradation.

3.3. Validation of the Method. The described method for the assay of TMH has been validated as per the current ICH Q2 (R1).

Table 6: Results of determination of TMH in tablet and statistical comparison with the reference method.

Formulation brand name	Nominal amount, mg	% TMH found* ± SD		t-value	F-value
		Reference method	Proposed method		
Nobligan[a]	50.0	101.6 ± 1.64	100.1 ± 0.65	1.94	6.36
Meridol[b]	50.0	101.8 ± 1.36	99.98 ± 0.86	2.52	2.50

[a] Marketed by Piramal HC Ltd, Mumbai, India.
[b] Unimarc India Ltd, Mumbai, India.
*Mean value of five determinations. Tabulated t-value at 95% confidence level is 2.78; Tabulated F-value at 95% confidence level is 6.39.

Table 7: Results of recovery study by standard addition method.

Tablet studied	TMH in tablet, μg mL^{-1}	Pure TMH added, μg mL^{-1}	Total found, μg mL^{-1}	Pure TMH recovered* (%TMH ± SD)
Nobligan	100.1	50.0	150.50	100.8 ± 1.98
	100.1	100.0	200.51	100.4 ± 0.98
	100.1	150.0	249.38	99.69 ± 0.97
Meridol	99.98	50.0	150.63	101.3 ± 0.80
	99.98	100.0	199.45	99.47 ± 0.57
	99.98	150.0	249.26	99.52 ± 0.34

* Mean value of five determinations.

3.3.1. Analytical Parameters. A calibration curve was obtained for TMH from 0.5% to 150% of its stock solution. A linear correlation was obtained between the peak area and the concentration in the range of 0.5–300 μg mL^{-1} TMH from which the linear regression equation was computed and found to be

$$y = 30.620x + 19.330, \qquad r^2 = 0.9999, \qquad (1)$$

where y is the mean peak area, x is the concentration of TMH in μg mL^{-1}, and r is the correlation coefficient. The LOD and LOQ values, slope (m), y-intercept (a), and their standard deviations are evaluated and presented in Table 1. These results confirm the linear relation between concentration of TMH and the peak areas as well as the sensitivity of the method.

3.3.2. Accuracy and Precision. The percent relative error which is an indicator of accuracy is ≤1.0 and is indicative of high accuracy. The calculated percent relative standard deviation (%RSD) can be considered to be satisfactory. The peak-area-based and retention-time-based RSD values were <1%. The results obtained for the evaluation of accuracy and precision of the method are compiled in Tables 2 and 3.

3.3.3. Robustness and Ruggedness. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. At the deliberate varied chromatographic conditions (flow rate, temperature, and mobile phase composition), the analyte peak %RSD, tailing factor, and theoretical plates remained closer to the actual values. The RSD values ranged from 0.1 to 1.3% resumes the robustness of the proposed method. In method ruggedness, different columns (same lot), at different day by different analysts were performed. The results are summarized in Tables 4 and 5.

3.3.4. Stability of the Solution. At the specified time interval, %RSD for the peak area obtained from drug solution stability and mobile phase stability were within 1%. This shows no significant change in the elution of the peak and its system suitability criteria (%RSD, tailing factor, and theoretical plates). The results also confirmed that the standard solution of drug and mobile phase were stable at least for 24 hours during the assay performance.

3.3.5. Selectivity. Selectivity of the method was evaluated by injecting the mobile phase, placebo blank, pure drug solution, and tablet extract. No peaks were observed for mobile phase and placebo blank and no extra peaks were observed for tablet extracts (Figures 4(a) and 4(b)).

3.3.6. Application to Tablet. A 200 μg mL^{-1} solution of tablets was prepared as per "*preparation of tablet extracts and assay procedure*" and injected in triplicate to the UPLC system. The mean peak area of the tablet extract for this concentration was found to be equivalent to that of pure drug solution of the same concentration and the results were compared with those of a reference method [4]. The accuracy and precision of the proposed method was further evaluated by applying Student's t-test and variance ratio F-test, respectively. The t- and F-values at 95% confidence level did not exceed the tabulated values and this further confirms that there is no significant difference between the reference and proposed methods with respect to accuracy and precision. Table 6 illustrates the results obtained from this study.

3.3.7. Recovery Study. A standard addition procedure was followed to further evaluate the accuracy of the method.

The solutions were prepared by spiking preanalyzed tablet with pure drug at three different levels and injected to chromatographic column. The recovery of the known amount of added analyte was computed. The percentage recovery of TMH from pharmaceutical dosage forms ranged from 99.4 to 101.34%. The results presented in Table 7 reveal good accuracy of the proposed method.

4. Conclusions

In this work, a stability indicating UPLC method was developed and validated for the determination of tramadol hydrochloride (TMH) in bulk and tablet dosage forms. The results of stress testing undertaken according to International Conference on Harmonization guidelines reveal that the method is selective and stability indicating which shows the applicability of the method for quality control. TMH was found to be more stable under thermolytic and photolytic stress conditions in the solid state rather than in acidic, basic, or aqueous stress condition in solution.

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