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Article

Development and Validation of a Stability-Indicating RP-UPLC Method for the Quantitative Analysis of Nabumetone in Tablet Dosage Form

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High efficiency and less run time are the basic requirements of high-speed chromatographic separations. To fulfill these requirements, a new separation technique, ultra-performance liquid chromatography (UPLC), has shown promising developments. A rapid, specific, sensitive, and precise reverse-phase UPLC method is developed for the determination of nabumetone in tablet dosage form. In this work, a new isocratic chromatographic method is developed. The newly developed method is applicable for assay determination of the active pharmaceutical ingredient. The chromatographic separation is achieved on a Waters Acquity BEH column (100 mm, i.d., 2.1 mm, 1.7 μm) within a short runtime of 2 min using a mobile phase of 5 mM ammonium acetate–acetonitrile (25:75, v/v), at a flow rate of 0.3 mL/min at an ambient temperature. Quantification is achieved with photodiode array detection at 230 nm, over the concentration range of 0.05–26 μg/mL. Forced degradation studies are also performed for nabumetone bulk drug samples to demonstrate the stability-indicating power of the UPLC method. Comparison of system performance with conventional high-performance liquid chromatography is made with respect to analysis time, efficiency, and sensitivity. The method is validated according to the ICH guidelines and is applied successfully for the determination of nabumetone in tablets.

Introduction

A new category of separation technique, ultra-performance liquid chromatography (UPLC), has proven to be one of the most promising developments in the area of fast chromatographic separations with its unique characteristics of high chromatographic resolution, speed, and sensitivity analysis (1–4). It utilizes sub-2 μm particles for the stationary phase. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in efficiency (5). In the present work, this technology has been applied to the method development, validation, and assay determination of nabumetone bulk drug.

Nabumetone is used for the treatment of pain or inflammation caused by rheumatoid arthritis, osteoarthritis, and acute soft tissue injuries (6). It is a non-acidic prodrug that is rapidly metabolized after absorption to a major active metabolite, 6-methoxy-2-naphthylacetic acid, which is responsible for its therapeutic efficacy (7). It has been shown to have a slightly lower risk of gastrointestinal side effects than most other non-selective non-steroidal anti-inflammatory drugs (NSAIDs) (8). A literature survey revealed high-performance liquid chromatography (HPLC) methods for the estimation of nabumetone in human plasma and pharmaceutical dosage forms (9–11). Simultaneous analysis of naproxen, nabumetone, and its major metabolite, 6-methoxy-2-naphthylacetic acid, is reported in pharmaceuticals and human urine by HPLC (12).

In this work, the HPLC method for nabumetone was transferred to UPLC. A comparison was made between HPLC and UPLC efficiency on the basis of resolution and sensitivity. The developed stability-indicating reversed-phase (RP) UPLC method was then validated as per ICH guidelines, along with the application of the developed method in the assay of marketed nabumetone tablets. However, there are no reports available on stability indicating RP-UPLC analytical method for nabumetone in tablet dosage form with short run time. It was, therefore, necessary to develop a new rapid stability-indicating method for the quantitative estimation of nabumetone in tablet dosage form. This work shows how the UPLC can reduce analysis times without compromising the resolution.

Experimental

Materials and reagents

Reference standard of nabumetone was received from Analytical Department, Ranbaxy Research Laboratories (Gurgaon, India) and tablets of Nilitis-1000 (1000 mg) of Ipca Laboratories Ltd. were purchased from a local pharmacy (India). The chemical structure of nabumetone is shown in Figure 1. HPLC-grade acetonitrile and ammonium acetate were purchased from Qualigens Fine Chemicals (India). High-purity water was obtained by Millipore Milli Q water purification system (Millipore, Billerica, MA).

HPLC

The HPLC system used for initial chromatographic development was Waters Alliance separation module with a 2996 UV detector (Waters, Milford Massachusetts). Chromatographic separation was achieved in both the modes (isocratic and gradient). A Kromasil C_{18}, 100 × 4.6 mm, 5 μm column was used for isocratic and Kromasil C_{18}, 250 × 4.6 mm, 5 μm column was used for gradient separation (Kromasil, Brewster, NY). A mobile phase consisting of a mixture of A: 20 mM ammonium acetate and B: acetonitrile in the ratio 25:75 (v/v) for isocratic mode while a timed gradient program T (min)/%B: 0/20, 5/20, 10/80, 15/80, 25/20, 30/20, with the flow rate of 1 mL/min was employed. The injection volume was 5 μL while detector was set at 230 nm. The column was maintained at 25°C.

UPLC

UPLC was performed using a Waters Acquity system equipped with binary solvent delivery pump, an auto sampler and tunable UV detector (Waters). The chromatographic separation was

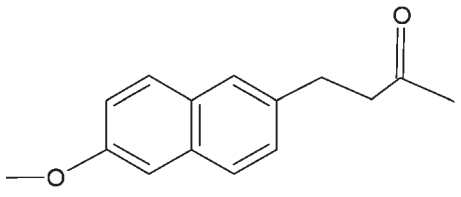

Figure 1. Structure of nabumetone.

performed using a Waters Acquity BEH 100 × 2.1 mm, 1.7 μm, C_{18} column (Waters). The mobile phase containing a mixture of 5 mM ammonium acetate and acetonitrile in the ratio of 25:75 (v/v) at a flow rate of 0.3 mL/min was used. The detection was obtained at a wavelength of 230 nm. The injection volume was 1 μL; water–acetonitrile (50:50 v/v) was used as a diluent while the column was maintained at 25°C.

Preparation of solution

A standard and test solution of nabumetone and Nilitis-1000 tablet (20 μg/mL each) were prepared using a diluent for assay determination. A standard stock solution of nabumetone (200 μg/mL) was prepared by dissolving 10 mg of drug in 50 mL water–acetonitrile (50:50 v/v). Working standard solution (20 μg/mL) was prepared from stock solution by proper dilution with water–acetonitrile (50:50 v/v) for system suitability.

The test solution (20 μg/mL) was prepared by dissolving appropriate amount of Nilitis-1000 in a diluent. Twenty tablets, each containing 1000 mg of Nabumetone, were weighed and finely powdered. A quantity of tablet powder equivalent to 1000 mg of nabumetone was transferred to a 250-mL volumetric flask, dissolved, and diluted with water–acetonitrile (50:50 v/v) to obtain 4 mg/mL. The solution was sonicated for 30 min, diluted to obtain a concentration of 200 μg/mL, and then diluted further to obtain a concentration of 20 μg/mL with water–acetonitrile (50:50 v/v).

Validation procedure

The newly developed UPLC method was validated in terms of precision, accuracy, and linearity according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (13). Assay method precision was carried out using six independent test solutions and a standard preparation. The intermediate precision of the assay method was also evaluated using different instruments on two different days. The accuracy of the assay method was evaluated in triplicate using three concentration levels: 16, 20, and 24 μg/mL. The linearity solutions for nabumetone were prepared by diluting the standard stock solution. The linearity study was also carried at the limit of quantification (LOQ) level. The limit of detection (LOD) and LOQ for nabumetone were estimated by injecting a series of dilute solutions with a known concentration. To determine the robustness of the method, experimental conditions were purposely altered, and the resolution of nabumetone was examined by injecting a system suitability solution. The flow rate was changed to 0.32 and 0.28 mL/min. The column temperature was varied by (±) 5°C (i.e., 20°C and 30°C). The organic strength was varied by (±) 2 mL absolute (i.e., 23:77 and 27:73).

Forced degradation studies of the tablet sample were also performed using the following conditions: acid hydrolysis (1 N hydrochloric acid), base hydrolysis (1 N sodium hydroxide), heat (68°C for 3 h), and oxidation (30% hydrogen peroxide). A peak purity test was carried out for the nabumetone peak by using a photodiode array (PDA) detector in stress samples.

Results and Discussion

LC method development and transfer to UPLC

The main target of the chromatographic method was to achieve separation and quantification of nabumetone with a short run time. Initially, the gradient and isocratic HPLC conditions were optimized for nabumetone in bulk drug, which was then transferred to UPLC. A gradient system is always preferred over an isocratic system in order to achieve improved peak shape and resolution. Hence, it was firstly decided to use a gradient HPLC mode. The chromatographic separation was achieved on a Kromasil C_{18}, 250 × 4.6 mm, 5 μm column maintained at 25°C. In gradient mode, using a mobile phase consisting of a buffer of 20 mM ammonium acetae and acetonitrile, there was a good separation of nabumetone, while it was found to be eluting at a higher retention time (around 14.6 min). To reduce the run time, it was decided to switchover to an isocratic HPLC mode with Kromasil C_{18}, 100 × 4.6 mm, 5 μm. With an isocratic system, less buffer and more organic strength was preferred, as a high composition of buffer can cause choking of the column. Moreover, a column of shorter length provides better separation and faster run times, because the smaller length gives less backpressure; thus, it can be operated at higher flow rates with faster gradients, while maintaining the same resolution.

The response of nabumetone was found to be adequate at 230 nm. The basic chromatographic conditions, such as the stationary phase, solvents, and UV detection employed in HPLC, were taken into account while developing the new UPLC method. The detection wavelength, column temperature, and solvent used in HPLC were kept constant. The C_{18} stationary phase was chosen in order to have similar chemistry to that used in the HPLC. In UPLC, an Acquity BEH C_{18}, 100 × 2.1 mm, 1.7 μm column was employed for the separation. The injection volume was scaled to 1 μL from 5 μL, as used in HPLC. An isocratic mode was chosen with the same ratio of buffer to acetonitrile as used in an isocratic HPLC mode (25:75, v/v). The flow rate was scaled to 0.3 mL/min. Using these conditions, a satisfactory separation was achieved, and the peak eluted at approximately 1.278 min, giving a total run time of 2 min. A backpressure of 6000 psi was observed.

Comparison study of chromatographic performance

Comparative data on chromatographic performance of HPLC (gradient and isocratic) and UPLC (isocratic) has been obtained by injecting a solution of nabumetone (20 μg/mL). The performance parameters of both the systems are shown in Table I. It is observed that the elution time of nabumetone in

Table I
A Comparison of System Performance of HPLC and UPLC for Nabumetone

Parameter	HPLC (gradient)	HPLC (isocratic)	UPLC (isocratic)
Elution time (min)	14.62	2.08	1.27
Flow rate (mL/min)	1.00	1.00	1.00
Injection volume (μL)	5.00	5.00	1.00
Run time (min)	30.00	5.00	2.00
Tailing Factor	1.50	1.20	1.40
USP Plate Count	46,771	3,326	9,860

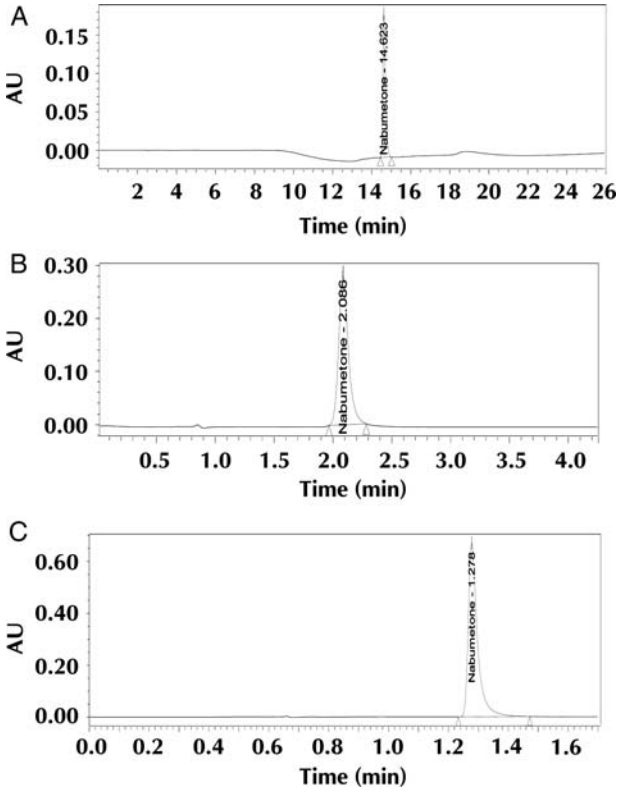

Figure 2. Comparison of the chromatograms of nabumetone obtained from (A) HPLC (gradient), (B) HPLC (isocratic), and (C) UPLC (isocratic).

Table II
Summary of Forced Degradation Studies

Stress condition	Time	Purity angle	Purity threshold	Remark
Acid hydrolysis (1 N HCl 5 mL at RT)	4 h	1.498	6.471	No degradation was found.
Base hydrolysis (1 N NaOH 5 mL at RT)	4 h	1.162	1.914	No degradation was found.
Oxidation (30% H_2O_2 5 mL heat)	0 h	11.937	38.562	No degradation was found.
Thermal (68°C)	4 h	0.560	0.925	No degradation was found.
Photostability	5 days	0.707	1.440	No degradation was found.

UPLC was reduced by 14-fold to that of a gradient mode HPLC and 2-fold to that of an isocratic mode HPLC. The resolution and theoretical plates obtained for nabumetone in UPLC show

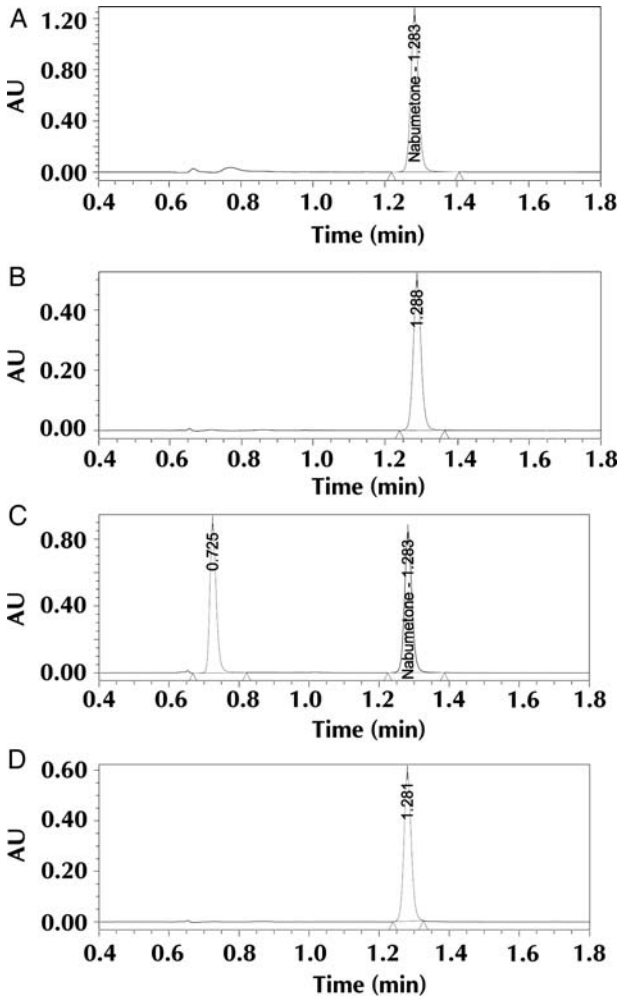

Figure 3. UPLC chromatograms of the nabumetone stressed samples: (A) acidic degradation, (B) basic degradation, (C) oxidative degradation, and (C) thermal degradation.

comparatively better separation efficiency than HPLC. Theoretical plates obtained for nabumetone in gradient mode HPLC was obviously higher, but in the case of an isocratic HPLC mode (eluting at 2.086 min), it is lesser than UPLC. The typical chromatograms obtained from the final HPLC (gradient, isocratic) and the UPLC conditions are depicted in Figure 2.

UPLC method validation

The validation study allowed the evaluation of the method for its suitability for routine analysis. The assay values of the tablet samples were found to be in the range of 96–99%. Forced degradation studies were also performed for nabumetone tablet sample to demonstrate the stability-indicating power of the newly developed UPLC method.

Specificity

Stress studies of the drug's active pharmaceutical ingredients were utilized for the identification of the possible degradation

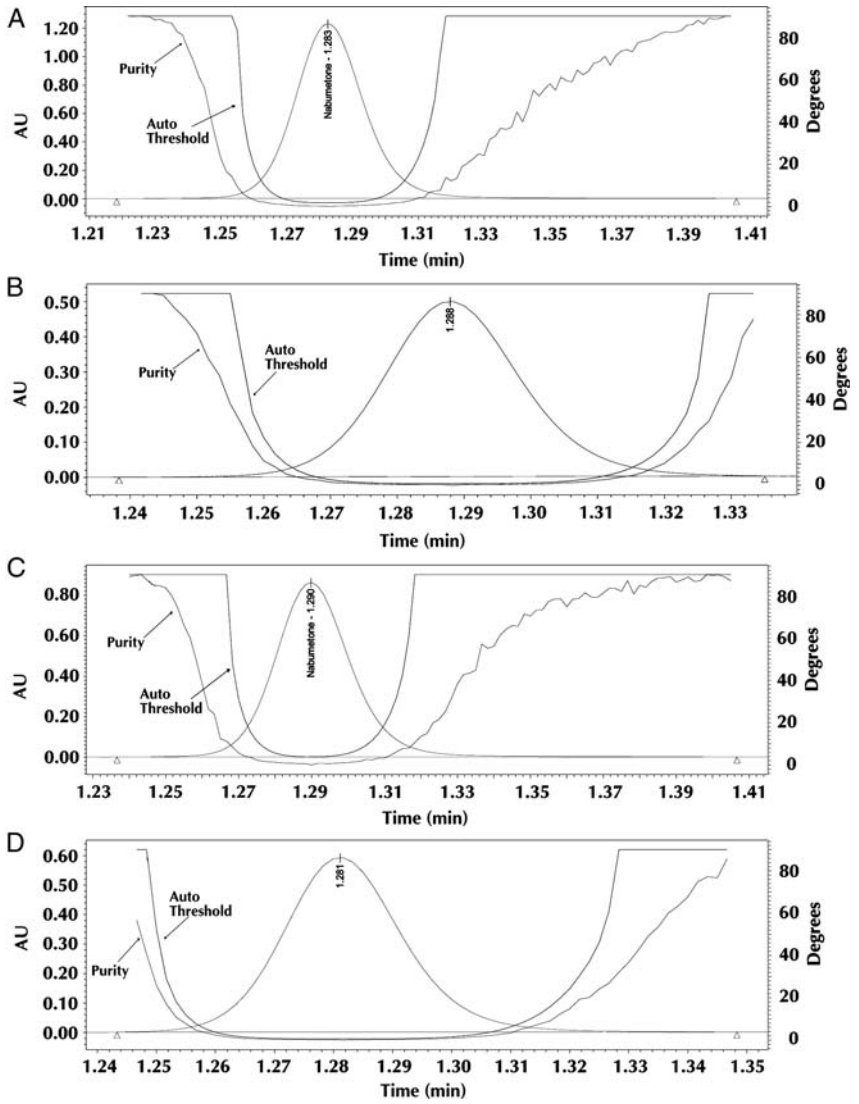

Figure 4. Photodiode array detection peak purity: (A) acidic degeneration, (B) basic degradation, (C) oxidative degradation, and (C) thermal degradation.

products and for the validation of the stability-indicating analytical procedures. It is the ability of the analytical method to measure the analyte response in the presence of its degradation products. The result obtained from the forced degradation studies is summarized in Table II. During the forced degradation study, the peak of Nabumetone was found to be pure and no considerable degradation of drug substance was observed in acidic, alkaline, thermal, and oxidative conditions. During analysis, a negligible amount of degradation had occurred, which is not considered relevant. Moreover, another peak at retention time 0.725 min was that of H_2O_2, which does not indicate the degradation of the analyte (Figure 3). The chromatograms were checked for the appearance of any extra or overlapping peaks. Peak purity of these samples under stressed conditions was verified using a PDA detector (Figure 4). The purity of the principle and other chromatographic peaks was found to be satisfactory. This study confirmed the stability indicating power of the UPLC method.

Table III
Recovery Analysis of Nabumetone

Conc. Level	Actual (μg/mL)	Recovered (μg/mL)	Recovery %	Mean
At 80% ($n = 3$)	15.20	13.96	91.80	93.39
	15.20	14.71	96.80	
	15.20	13.92	91.60	
At 100% ($n = 3$)	18.80	18.24	97.00	96.24
	18.80	18.16	96.60	
	18.80	17.88	95.10	
At 120% ($n = 3$)	22.32	21.72	97.30	96.18
	22.32	21.27	95.30	
	22.32	21.42	96.00	

Precision

The precision of the assay method was evaluated by carrying out six independent assays. The relative standard deviation (RSD) of the assay of nabumetone determination was within the acceptable limit of 2%. The precision of tablet sample was

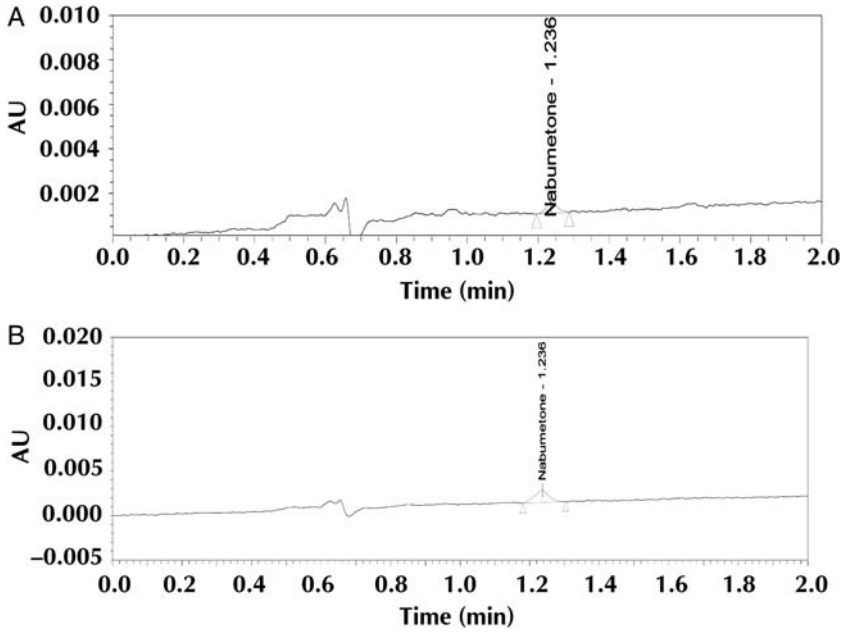

Figure 5. The LOD (A) and LOQ (B) of nabumetone demonstrated by UPLC.

examined using six replicate injections of the test solution. The RSD for the tablet sample was found to be 0.95%, respectively. These values are well within the generally acceptable limit.

The RSD of the assay results obtained in the intermediate precision study was within 2%, and the RSD of the response for the tablet sample was found to be 1.39%, confirming good precision of the assay.

Accuracy

The accuracy of the method was determined for the nabumetone by spiking the stock solution of nabumetone in a blank matrix in triplicate at levels 80%, 100%, and 120% of the specified limit. The recoveries of nabumeone were calculated and given in Table III. The accuracy of the assay method was evaluated in triplicate at three concentration levels, 16, 20, and 24 μg/mL in the bulk drug sample. The percentage recovery of nabumetone in the bulk drug samples ranged from 91.6 to 97.3%, respectively. High recovery results obtained from the proposed UPLC assay method indicates that this method can be used for quantitative routine quality control analysis of pharmaceutical dosage form.

LOD and LOQ

The LOD and the LOQ of the drug were found by observing the solution of nabumetone as having different lower concentrations, and the LOD and LOQ were found to be 0.01 and 0.05 μg/mL, indicating that the method is sensitive (Figure 5).

Linearity

Linear calibration plots for nabumetone were obtained over the calibration range (LOQ to 26 μg/mL) at 11 concentration levels in duplicate. The corresponding regression equation was $y = 68897x - 15724$, with the correlation coefficient (R^2) greater than 0.998. The results showed excellent correlation between the peak area and concentration of nabumetone (Table IV).

Table IV
Results of Regression Analysis of Linearity Data of Nabumetone Bulk Drug

Parameters	Nabumetone
Linearity range (μg/mL)	0.05–26
Correlation coefficient (r^2)	0.9989
Slope (m)	68897
Intercept (c)	−15724
LOD (μg/mL)	0.01
LOQ (μg/mL)	0.05
Accuracy (%)	91.6–97.3%
Repeatability (RSD, %, $n = 6$)	0.151
Inter-day Precision (RSD, %, $n = 6$)	1.398%

Robustness

To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, column temperature, and UV detector wavelength). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were evaluated for the studies. In all the deliberately varied chromatographic conditions, the chromatogram for system suitability solution showed satisfactory resolution (RSD $< 2\%$) with no significant changes in chromatographic parameters (Table V).

Sensitivity of UPLC

The LOQ concentration was found to be 0.05 μg/mL, with an RSD of 4.5%. The LOD concentration was found to be 0.01 μg/mL, with an RSD of 18.1% at an injection volume of 1 μL. The

Table V
Robustness Evaluation of the Developed UPLC Method

Chromatographic changes	%RSD (Peak Area) $n = 2$*	Tailing factor	Theoretical plate count
Flow rate (mL/min)			
0.28	0.12%	1.30	7105
0.3†	0.10%	1.40	6719
0.32	0.54%	1.30	5776
Temperature (°C)			
20	0.15%	1.60	9429
25†	0.10%	1.40	6719
30	0.43%	1.60	9325
UV Wavelength			
228	0.03%	1.70	9841
230†	0.10%	1.40	6719
232	0.15%	1.70	9843
Mobile phase composition (mL)			
23:77	0.42%	1.60	8811
25:75†	0.10%	1.40	6719
27:73	0.19%	1.30	7475

* All values given are taken as mean of 2 individual values.
† Optimum condition.

lower LOQ values with higher precision indicate better sensitivity of the UPLC method.

Conclusion

The newly developed stability indicating RP-UPLC method for assay determination of nabumetone was found to be capable of giving faster retention times, while maintaining good resolution than that achieved with conventional HPLC. The method was completely validated, showing satisfactory data for all the parameters tested. This method exhibited an excellent performance in terms of sensitivity and speed. It is a stability-indicating method suitable for the rapid analysis of a nabumetone bulk drug and its tablets.

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