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Research Article

Development and Validation of Stability-Indicating RP-UPLC Method for the Determination of Methdilazine in Bulk Drug and in Pharmaceutical Dosage Form

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A simple, precise, and accurate, and stability-indicating isocratic Ultraperformance Liquid Chromatography (UPLC) method was developed for the determination of methdilazine hydrochloride (MDH) in bulk drug and in its tablets. The use of 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 MDH, is demonstrated. The method was developed using Waters Acquity BEH C18 column (100 mm $\times$ 2.1 mm, 1.7 μm) with mobile phase consisting of a mixture of potassium dihydrogenorthophosphate and 1-pentane sulphonic acid buffer of pH 4.0 and acetonitrile (60 : 40 v/v). The eluted compound was detected at 254 nm with a UV detector. The standard curve of mean peak area versus concentration showed an excellent linearity over a concentration range 0.5–80 μg mL^{-1} MDH with regression coefficient (r) value of 0.9999. The limit of detection ($S/N = 3$) was 0.2 μg mL^{-1} and the limit of quantification ($S/N = 10$) was 0.5 μg mL^{-1}. Forced degradation of the bulk sample was conducted in accordance with the ICH guidelines. Acidic, basic, hydrolytic, oxidative, thermal, and photolytic degradations were used to assess the stability indicating power of the method. The drug was found to be stable in acidic, basic, thermal, hydrolytic, and photolytic stress conditions and showed slight degradation in oxidative stress condition.

1. Introduction

Methdilazine hydrochloride (MDH), chemically known as (10-[(1-Methyl-3 pyrrolidinyl)methyl]phenothiazine monohydrochloride) [1] (Figure 1), is a synthetic analogue of phenothiazine derivative used as an antihistamine and it is also found to possess antipruritic action [2].

The drug is official in United States Pharmacopia [3], which describes UV-spectrophotometric assay in aqueous medium. The literature survey revealed the availability of few methods for the assay of MDH in pharmaceutical formulations. Quantification of MDH has been achieved by high-performance liquid chromatography (HPLC) [4–6], liquid chromatography [7], spectrofluorimetry [8], differential fluorimetry, and differential UV-spectrophotometry [9]. Some visible spectrophotometric methods are also reported for the assay of on pharmaceuticals [10–22].

In recent years, there has been an increasing tendency towards development of stability-indicating assays [23–26], using the approach to stress testing enshrined in International Conference on Harmonisation (ICH) guideline Q2A(R2) [27]. This approach is being extended to pharmaceuticals to enable accurate and precise quantification of drugs in the presence of their degradation products.

Ultraperformance liquid chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption [28]. UPLC system is designed in a special way to withstand high system back-pressure. 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 analyses time up to nine times compared to the conventional HPLC system, but separation efficiency remains the same or is even improved.

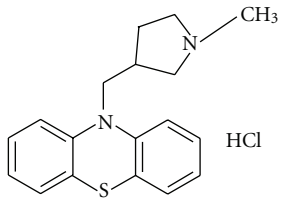

Figure 1: Structure of MDH.

As efficiency and speed of analyses are of great importance in many applications of liquid chromatography, especially in pharmaceutical, toxicological, and chemical analyses, where it is important to increase throughput and reduce analyses costs, UPLC could play a significant role in the future of liquid chromatography.

Though a number of liquid chromatographic methods have earlier been proposed for MDH [4–7], no attempt has been made to apply this new type of LC (UPLC) for pharmaceuticals 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 methdilazine HCl in bulk and tablets. This was accomplished with a Waters Acquity UPLC system and Acquity BEH column (C-18, 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. Pure active ingredient sample of was kindly supplied by Glaxo laboratories, Mumbai, India, as gift. Dilosyn 8 mg tablet (Glaxosmithkline Pharmaceuticals Ltd.) was purchased from local commercial sources. HPLC grade acetonitrile was purchased from Merck India, Mumbai, India and potassium dihydrogenorthophosphate and sodium pentane sulphonic acid were from Qualigens, India. Doubly distilled water was used throughout the investigation.

2.2. Chromatographic Conditions and Equipments. Analyses were carried out on a Waters Acquity UPLC with Tunable UV (TUV) detector. The output signal was monitored and processed using Empower software. The Chromatographic column used was Acquity UPLC BEH C-18 (100×2.1) mm and 1.7 μm particle size. Isocratic elution process was adopted throughout the analyses.

2.3. Mobile Phase Preparation. About 1.3 g of potassium dihydrogenorthophosphate and 1.1 g sodium pentane sulphonic acid was dissolved in 1000 mL of water and pH was adjusted to 4.0 using 10% orthophosphoric 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 0.25 mL min^{-1}. The column temperature was adjusted to 35°C. The injection volume was 2 μL. Eluted sample was monitored at 254 nm and the run time was 5.0 min. The retention time of the sample was about 2.3 min.

2.5. Stress Study. All stress decomposition studies were performed at an initial drug concentration of 40 μg mL^{-1} in mobile phase and added 5 mL of 5 M HCl, 5 M NaOH, or 5% H_2O_2 separately, and the flasks were heated for 2 h on a water bath maintained at 80°C. Then the solutions were cooled and neutralized by adding base or acid, the volume in each flask was brought to the mark with mobile phase, and the appropriate volume (2.0 μL) was injected for analyses. Solid-state thermal degradation was carried out by exposing pure drug to dry heat at 105°C for 3 h. For photolytic degradation studies, pure drug in solid state was exposed to 1.2 million lux hours in a photo stability chamber. The sample after exposure to heat and light was used to prepare 40 μg mL^{-1} solutions in mobile phase and the chromatographic procedure was followed.

2.6. Preparation of Stock Solution. A stock standard solution of (1 mg mL^{-1}) was prepared by dissolving an accurately weighed 100 mg of pure drug in 100 mL volumetric flask using the mobile phase.

2.7. Procedures

2.7.1. Procedure for Preparation of Calibration Curve. Working solutions containing 0.5–80 μg mL^{-1} of MDH were prepared by serial dilutions of aliquots of the stock solution. Aliquots of 2.0 μL were injected in triplicate (six injections) and eluted with the mobile phase under the reported chromatographic conditions. The average peak area versus the concentration of MDH in μg mL^{-1} was plotted. Alternatively, the corresponding 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 Dilosyn-8 tablets (each tablet contained 8.0 mg) were weighed and transferred into a clean, dry mortar and powdered. Tablet powder equivalent to 4 mg of MDH was transferred into a 100 mL volumetric flask and 60 mL of the mobile phase was added. The solution was sonicated for 20 min to achieve complete dissolution of MDH, made up to the mark with mobile phase, and then filtered through a 0.22 μm nylon membrane filter. The solution (40 μg mL^{-1} of MDH) obtained was analyzed by UPLC.

2.7.3. Procedure for Method Validation

Accuracy and Precision. To determine the accuracy and intraday precision, pure MDH solutions at three different concentrations were analyzed in seven replicates during the same day. Mobile phase was injected as blank solution before

sample injection and the RSD (%) values of peak area and retention time were calculated.

Limits of Detection (LOD) and Quantification (LOQ). The LOD and LOQ were obtained by signal to noise (S/N) ratio method. LOQ and LOD were obtained by a series of dilutions of the stock solution. Precision study was performed at LOQ level also. LOQ solution was injected seven times ($n = 7$) and calculated the % RSD values for the obtained peak area and retention time.

Linearity. Linearity solutions were prepared from LOQ level to 200% of the actual sample concentration ($40\,\mu g\,mL^{-1}$). A total of six concentrations of the solutions were made separately and injected (LOQ, 20, 40, 60, and $80\,\mu g\,mL^{-1}$ levels).

Robustness and Ruggedness. To determine the robustness of the method, the experimental conditions were deliberately changed. The flow rate of the mobile phase ($0.25 \pm 0.05\,mL\,min^{-1}$), column oven temperature ($35 \pm 5^{\circ}C$), mobile phase composition (65 : 35, 60 : 40 and 55 : 45 buffer : acetonitrile v/v), and detection wavelength (254 ± 1 nm) were the varied parameters. In each case the % RSD values was 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 on the same day and on three different days by three different analysts for three different concentrations of MDH (triplicates injection). The area obtained from each concentration was compared with that of the optimized one. The relative standard deviation values were evaluated for each concentration.

Solution Stability and Mobile Phase Stability. Stability of solution was investigated by injecting the sample into the chromatographic system. The peak area was recorded in the time intervals of 0, 12, and 24 hrs and the RSD values were calculated. The mobile phase stability was studied by injecting a freshly prepared sample solution at the same time intervals (0, 12, and 24 hours) with the same mobile phase and RSD values of the peak areas were calculated.

3. Results and Discussion

3.1. Method Development. UPLC becomes very prominent in recent years due to its fast approach towards drug method development and validation. The smaller particles in column provide not only increased efficiency, but also the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. The present method discusses in detail the development and validation of MDH with vital information about its degradation under different stress conditions.

Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer and solvents 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. Alternative combinations of gradient and isocratic methods were also performed to obtain a suitable peak. Finally, isocratic method was found suitable for the assay.

Table 1: Summary of solvent optimization.

Solvent A	Solvent B	Observations/remarks
5 mM ammonium acetate	Methanol	Very broad peak with peak splitting
5 mM ammonium acetate	Acetonitrile	Peak eluted too early
Dibasic potassium phosphate (pH 3.0 with 10% H_3PO_4)	Methanol	Very broad peak with peak splitting
Dibasic potassium phosphate (pH 3.0 with 10% H_3PO_4)	Acetonitrile	Less theoretical plates and peak fronting
Monobasic potassium phosphate (pH 3.0 with 10% H_3PO_4)	Methanol	Broad peak and less theoretical plates
Potassium dihydrogen orthophosphate and 1-pentane sulphonic acid (pH 4.0) at 60% ratio	Acetonitrile at 40% ratio	Good peak shape with theoretical plates above 2000

When MDH was injected with methanol and potassium phosphate buffer mobile phases, the resultant peak showed either tailing or much shortened retention time (less than 1 min). As the buffer ratio increased, the retention time of the MDH augmented but peak eluted with abnormal shape. The concentration of buffer and methanol varied in different ratios and was found incompatible. The separation was carried out with Acquity BEH C18, (100×2.1) mm, $1.7\,\mu$m column. Acetonitrile was the next solvent option. Better results were obtained when MDH was eluted in acetonitrile, and potassium dihydrogenorthophosphate and 1-pentane sulphonic acid buffer. MDH eluted late with higher peak shape and theoretical plates. The peak symmetry was optimized with varying concentrations of buffer and acetonitrile. The best peak was obtained with buffer-acetonitrile ratio (60 : 40 v/v). Different buffers like ammonium acetate and dibasic potassium phosphate were the other salts used for development. Flow rate of 0.25 mL/min was selected with regard to the backpressure and analyses time as well. The solvents used are summarized in Table 1.

In order to achieve symmetrical peak of MDH, various stationary phases like C8 (different dimension), C18 (different brand), length (50 mm and 100 mm), and phenyl columns were studied. Summary of stationary phases is represented as in Table 2. From the summary, it is concluded that Acquity BEH C18, (100×2.1) mm, $1.7\,\mu$m column has the ideal stationary phase for the determination. The column oven temperature was studied at higher ($40^{\circ}C$) and room ($25^{\circ}C$) temperatures and then found that $35^{\circ}C$

Table 2: Summary of stationary phase optimization.

Stationary phase	Dimension	Observations/remarks
Acquity BEH Phenyl	(100 × 2.1) mm, 2 μm	Peak eluted very early with higher tailing
Acquity BEH C8	(50 × 2.1) mm, 1.7 μm	No retention of MDH, eluted very early
Eclipse Plus C18, RRHD	(50 × 2.1) mm, 1.8 μm	Peak fronting observed
Acquity BEH C8	(100 × 2.1) mm, 1.7 μm	Peak eluted before 1 min
Acquity BEH C18	(100 × 2.1) mm, 1.7 μm	Satisfactory peak shape observed

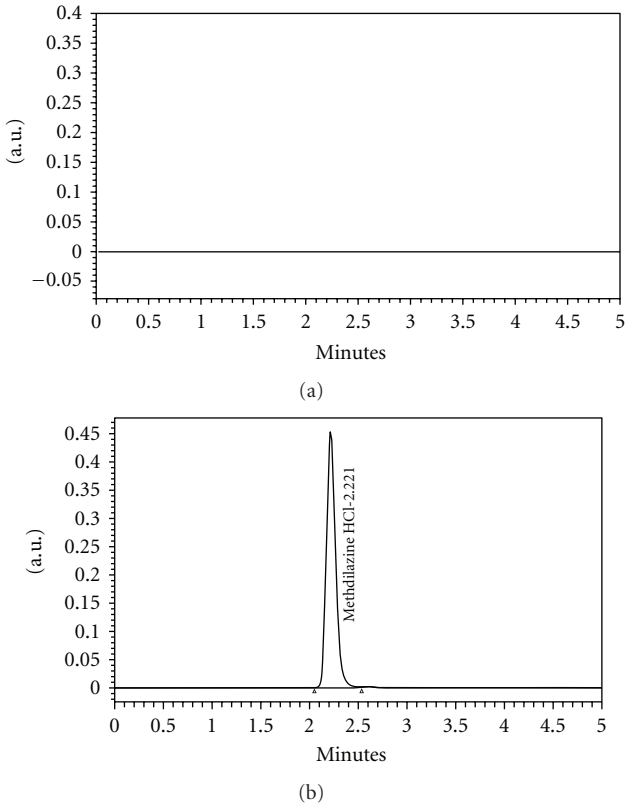

Figure 2: (a) Blank and (b) chromatogram of MDH (40 μg mL^{-1}).

is the optimum. Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer was used for absorbance measurements. A 40 μg mL^{-1} of MDH solution in acetonitrile was scanned from 400 to 200 nm against acetonitrile as blank and wavelength of the method was optimized to 254 nm. Overlay chromatograms of blank and MDH solutions are as shown in Figure 2.

3.2. Stability Studies. All forced degradation studies were analyzed at 40 μg mL^{-1} concentration level. The observation was made based on the peak area of the respective sample. MDH was found to be more stable under acid and alkaline, 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. Less degradation occurred under oxidative stress conditions with percent decomposition being only around 10%. The chromatograms that obtained for MDH after subjecting to degradation are presented in

Table 3: Linearity and regression parameters with precision data.

Parameter	Value
Linear range, μg mL^{-1}	0.5–80
Limits of quantification, (LOQ), μg mL^{-1}	0.5
Limits of detection, (LOD), μg mL^{-1}	0.2
Regression equation	
Slope (b)	71371.1
Intercept (a)	43683.5
Correlation coefficient (r)	0.9999

Table 4: Results of accuracy study ($n = 5$).

Concentration of MDH injected, μg mL^{-1}	Intraday		Interday	
	Concentration of MDH found, μg mL^{-1}	RE, %	Concentration of MDH found, μg mL^{-1}	RE, %
20.0	20.15	0.75	20.19	0.95
40.0	40.24	0.61	40.35	0.87
60.0	59.73	0.45	59.58	0.70

Figure 3. Assay study was carried out by the comparison with the peak area of MDH sample without degradation.

4. Analytical Parameters Validation

The described method for the assay of MDH was validated as per the current ICH guidelines. Parameters such as system suitability, specificity, precision, robustness, ruggedness, linearity, accuracy, LOQ, LOD, solution stability, and filter compatibility were studied for the suitability of the method.

4.1. System Suitability. System suitability is the measurement of performance verification of system, method, and column performance. Theoretical plates (should be more than 2000), tailing factor (should be less than 1.5), and percentage relative standard deviation (should be less than 2) for the area and retention time of replicate injections were verified on precision, ruggedness (variation in column, analyst and day), and robustness (variation in temperature, mobile phase and wavelength) of the validation. As seen from the data, the theoretical plates found are more than 2000, tailing factor is less than 1.4, and the percentage relative standard deviation (% RSD) for area and retention time was less than 0.5.

4.2. Analytical Parameters. A calibration curve was obtained for MDH from LOQ to 200% of its stock solution. A linear

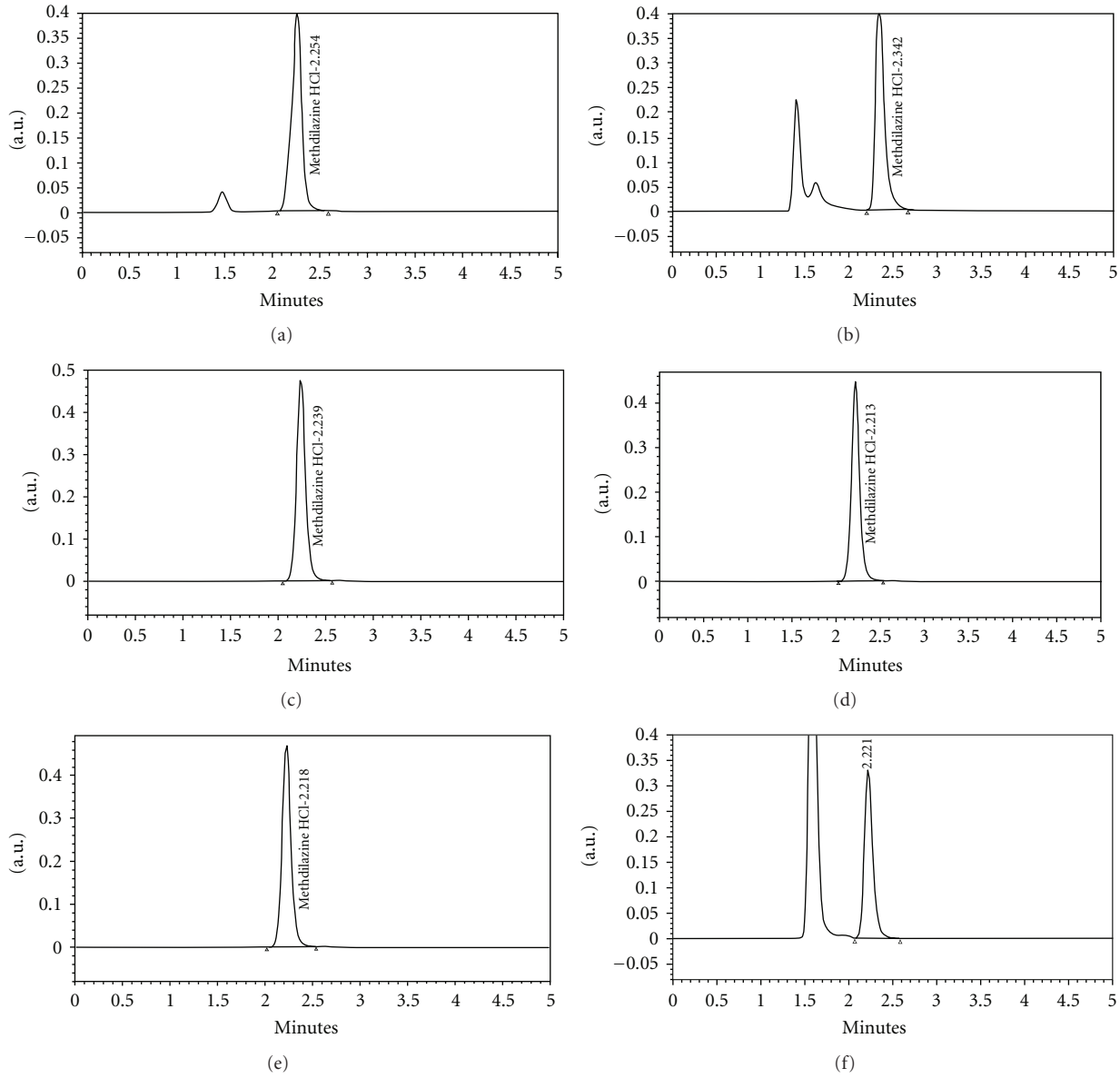

Figure 3: Chromatograms of MDH after forced degradation (40 μg mL^{-1}) (a) acid degradation, (b) base degradation, (c) hydrolytic degradation, (d) thermal degradation, (e) photolytic degradation, and (f) oxidative degradation.

Table 5: 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]
20.0	1456560 ± 6277	0.43	0.31	1430424 ± 6948	0.48	0.32
40.0	2976574 ± 11058	0.37	0.24	2912796 ± 11769	0.40	0.28
60.0	4380383 ± 6228	0.14	0.13	4319865 ± 6987	0.16	0.17

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

correlation was obtained between the mean peak area and the concentration in the range of 0.5–80 μg mL^{-1} CPH from which the linear regression equation was computed and found to be

$$y = 71,371x + 43,683 \; r^2 = 0.9999, \quad (1)$$

where y is the mean peak area, x is the concentration of 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 3. These results confirm the linear relation between the mean peak

Table 6: Method robustness.

Condition	Modification	Mean peak area ± SD*	RSD, %	Mean R_t ± SD*	RSD, %	Mean theoretical plates ± SD*	RSD, %	Mean tailing factor ± SD*	RSD, %
Temperature	30°C	2997425 ± 3368	0.11	2.226 ± 0.005	0.21	2702 ± 59.65	2.20	1.25 ± 0.01	0.80
	35°C	2932419 ± 6736	0.22	2.224 ± 0.003	0.14	2767 ± 39.36	1.42	1.23 ± 0.02	1.62
	40°C	2947846 ± 5823	0.19	2.222 ± 0.004	0.18	2786 ± 47.56	1.70	1.28 ± 0.02	1.56
Mobile phase composition	65 : 35	2943821 ± 5638	0.19	2.220 ± 0.006	0.26	2689 ± 53.36	1.98	1.32 ± 0.02	1.62
	60 : 40	2891630 ± 4178	0.14	2.221 ± 0.005	0.24	2772 ± 32.56	1.17	1.27 ± 0.02	1.57
	55 : 45	2985915 ± 6281	0.21	2.216 ± 0.005	0.25	2865 ± 55.97	1.95	1.35 ± 0.01	0.74
Flow rate, min	0.20	2915998 ± 5246	0.18	2.224 ± 0.005	0.21	2805 ± 48.16	1.71	1.29 ± 0.02	1.55
	0.25	2908856 ± 2857	0.10	2.219 ± 0.004	0.18	2698 ± 42.84	1.58	1.30 ± 0.01	0.76
	0.30	2991556 ± 6649	0.22	2.221 ± 0.003	0.14	2791 ± 53.16	1.90	1.22 ± 0.02	1.63
Wavelength	253 nm	2906684 ± 4479	0.15	2.228 ± 0.008	0.35	2695 ± 35.16	1.30	1.34 ± 0.02	1.49
	254 nm	3042479 ± 3805	0.09	2.215 ± 0.006	0.27	2747 ± 42.3	1.53	1.32 ± 0.02	1.51
	255 nm	2944213 ± 8138	0.28	2.224 ± 0.004	0.17	2698 ± 38.58	1.42	1.28 ± 0.02	1.96

* Mean value of three determinations.

Table 7: Method ruggedness.

Variable	Mean Peak area ± SD*	RSD, %	Mean R_t ± SD*	RSD, %	Mean theoretical plates ± SD*	RSD, %	Mean tailing factor ± SD*	RSD, %
Analyte ($n = 3$)	2976574 ± 11058	0.37	2.226 ± 0.005	0.21	2783 ± 54.34	1.95	1.24 ± 0.02	1.61
Column ($n = 3$)	2991556 ± 6649	0.22	2.220 ± 0.006	0.26	2804 ± 59.78	2.13	1.26 ± 0.03	2.38

* Mean value of three determinations.

area and concentration as well as the sensitivity of the method.

4.3. Accuracy and Precision. The percent relative error which is an index of accuracy is ≤1.5 and is indicative of high accuracy. The calculated percentage relative standard deviation (% RSD) can be considered to be satisfactory. The peak area based and retention time based RSD values were <1. The results that obtained for the evaluation of precision and accuracy of the method are compiled in Tables 4 and 5.

4.4. 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 near to the actual values. The RSD values that ranged from 0.1 to 2.0% resume the robustness of the proposed method. In method ruggedness, different columns (same lot), on different days by different analysts were performed. The results were summarized in Tables 6 and 7.

4.5. Stability of the Solution. At the specified time interval, % RSD for the peak area obtained from drug solution stability and mobile phase stability was within 1%. This shows no significant change in the elution of the peak and its system suitability criteria (% RSD, tailing factor, 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.

Table 8: Solution stability results.

	Initial	6 hours	12 hours	18 hours	24 hours
% Assay for solution stability	99.8	100.1	99.6	99.6	99.3
% Assay for mobile phase stability	99.7	100.0	100.1	99.8	99.7

4.6. 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 (Figure 4).

4.7. Stability of the Solution and Mobile Phase Stability. At the specified time interval, % assay of MDH obtained from drug solution stability and mobile phase stability was within 1%. For solution stability the same sample solution was injected at 0, 6, 12, and 24 hours and for mobile phase stability, separately prepared MDH sample was injected at the same time interval as above. The results confirmed that the MDH solution of drug and mobile phase were stable at least for 24 hours during the assay performance, which are represented in Table 8.

Table 9: Results of determination of MDH in tablet and statistical comparison with the reference method.

Formulation brand name	Nominal amount, mg	% MDH found* ± SD		t value	F value
		Reference method	Proposed method		
Dilosyn[a]	8.0	100.17 ± 0.61	100.38 ± 0.58	1.10	1.10

[a] Glaxosmithkline Laboratories LTD.
*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 10: Results of recovery study by standard addition method.

Tablet studied	MDH in tablet, μg mL^{-1}	Pure MDH added, μg mL^{-1}	Total found, μg mL^{-1}	Pure MDH recovered* (% MDH ± SD)
Dilosyn	30.05	15.0	45.28	100.51 ± 1.08
	30.05	30.0	60.44	100.64 ± 0.97
	30.05	45.0	74.76	99.61 ± 0.83

* Mean value of three determinations.

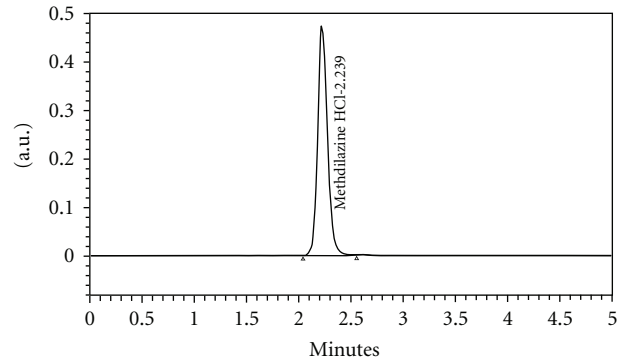

Figure 4: Chromatogram for 40 μg mL^{-1} dilosyn tablet extract.

4.8. Application to Tablet. A 40 μg mL^{-1} solution of tablets was prepared as per Section 2.7.2 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 [3]. The reference method consisted of measurement of the absorbance of aqueous solution of MDH at 252 nm. The accuracy and precision of the proposed method were 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 9 illustrates the results obtained from this study.

4.9. Recovery Study. A standard addition procedure was followed to evaluate the accuracy of the method. The solutions were prepared by spiking preanalyzed tablet powder with pure MDH at three different levels and injected to chromatographic column after sample preparation as described before. The recovery of the known amount of added analyte was computed. The percentage recovery of MDH from pharmaceutical dosage forms ranged from 99.61% to 100.64%. Detailed results presented in Table 10 reveal good accuracy of the proposed method.

5. Conclusion

A stability-indicating reverse phase UPLC method was developed and validated for the determination of MDH in n bulk drug and in tablets. Stress testing is an important aspect of the drug development process. The experimental design describes the scouting of the key UPLC method components including column, pH, and mobile phase. Their interrelationships are studied and the preliminary optimized conditions are obtained for each combination of column, pH, and mobile phase. Here, a better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the methods to meet their intended purposes is done. Moreover, this approach provides a thorough knowledge and enables the creation of a chromatographic database that can be utilized to provide alternative method conditions at a future time should changes to the method be required for both forms of the drug. The new method boosts productivity by providing more information per unit of work as UPLC swear higher resolution, speed, and sensitivity predicted for liquid chromatography. All the validated parameters were found within acceptance criteria. The validated method is specific, linear, precise, accurate, robust, rugged, and stable for 24 hours and can be applied for the determination in formulated form. 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 was found to be more stable under all subjected stress conditions other than in oxidative stress condition. The present method is superior over previously reported chromatographic/spectrophotometric methods with good accuracy and precision and specially concerning in decrease of time and solvent consumption. This method can be used as a general method for the determination of MDH in bulk powder and tablets. Hence, the method can be used in routine analyses of drug in quality control laboratories.

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