

STABILITY INDICATING RP-HPLC ASSAY METHOD FOR ESTIMATION OF MIDODRINE HYDROCHLORIDE IN BULK AND TABLETS

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Received: 22 Jun 2016 Revised and Accepted: 22 Jul 2016

ABSTRACT

Objective: To develop an accurate, simple, sensitive and precise stability indicating reverse phase-high performance liquid chromatographic (RP-HPLC) assay method for estimation of Midodrine hydrochloride (MD) in bulk and tablets.

Methods: The chromatographic separation was performed on enable C₁₈, (250 mm X 4.6 mm, 5 μm) column. The mobile phase consists of triethylamine buffer 0.02%, pH-3: acetonitrile (38:62 v/v) was delivered at a flow rate of 0.6 ml/min and UV detection at 289 nm. The method was validated with forced degradation studies as per ICH guidelines.

Results: The retention time of the drug was found to be 3.56 min. The developed method was found to be linear in a concentration range of 19.98-99.9 μg/ml of the drug (r²= 0.9998). The low value of % RSD indicates reproducibility of the method. The low value of LOD and LOQ suggests the sensitivity of the method. The results of forced degradation studies indicated that the drug was stable in acidic condition and degraded in basic, oxidative and hydrolytic conditions.

Conclusion: The present study represents first stability-indicating HPLC assay method that deals with the estimation of midodrine hydrochloride. It can be concluded from the results that the developed method is simple, rapid, accurate, specific, sensitive and precise. Thus, this method can be used for routine analysis of midodrine hydrochloride formulation and to check the stability of bulk samples.

Keywords: Midodrine hydrochloride, RP-HPLC, Method validation, Stability indicating assay method

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DOI: <http://dx.doi.org/10.22159/ijpps.2016v8i9.13619>

INTRODUCTION

Midodrine hydrochloride (MD) is chemically (±)-2-amino-N-(β-hydroxy-2, 5-dimethoxyphenethyl) acetamide monohydrochloride [1]. Midodrine is a prodrug, which forms an active metabolite desglymidodrine. Desglymidodrine is an alpha-1 agonist, activates α-adrenergic receptors of the arteriolar and venous vasculature that increases vascular tone and blood pressure [2]. This drug is official in United States Pharmacopeia (USP). Literature survey revealed that spectrophotometric methods for determination of midodrine hydrochloride [3, 4] and ion selective method [5] and HPLC [6]. Midodrine hydrochloride was also determined in plasma by HPLC method with fluorescence detection [7] and LC-MS/MS method [8]. There has no stability-indicating HPLC assay method reported yet for estimation of midodrine hydrochloride in bulk and pharmaceutical formulation. Therefore, the present work involves the development of a stability indicating RP-HPLC assay method for estimation of this drug in bulk and tablets.

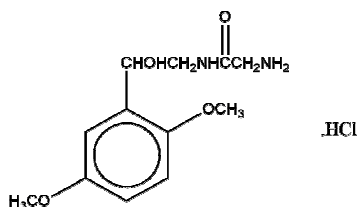


Fig. 1: Chemical structure of midodrine hydrochloride

MATERIALS AND METHODS

Chemicals and reagents

Midodrine hydrochloride was purchased from Industriale Chimica SRL, Italy. Commercially available tablets (ProAmatine®: 2.5 mg of

midodrine hydrochloride) used in this study were purchased from local market. HPLC grade of solvents was procured from Loba Chemie Pvt. Ltd., Mumbai and AR grade of chemicals were obtained from Merck Pvt. Ltd., Mumbai.

Instruments

The method was performed on shimadzu (LC 2010 CHT, Japan) having a quaternary system with automatic injection facility and UV-visible detection system. The column 'enable' C₁₈, LC solution software and shimadzu AY-120 balance were used for this work.

Preparation of standard stock solution

An accurately weighed quantity of drug midodrine hydrochloride equivalent to 100 mg was transferred to 100 ml volumetric flask. The drug was dissolved and diluted up to the mark with water.

Assay of tablets

Twenty tablets were weighed, and average weight was calculated. These tablets were crushed in a glass mortar. An accurately weighed 100 mg drug was transferred to a 100 ml of volumetric flask then diluted up to mark with water. The solution was filtered through whatman filter paper no. 41. This solution was further diluted to obtain 60 μg/ml solutions with water, and this procedure was repeated in triplicate [9, 10]. The results of the assay of tablets are shown in table 2.

Validation of the method

The developed chromatographic method was validated for system suitability, linearity, range, accuracy, precision and robustness parameters, as per ICH guidelines [11-14].

Linearity and range

Working standard solutions were injected under the optimized chromatographic conditions and peak areas were calculated at 289 nm. The calibration curve was plotted between areas against corresponding concentrations of the drug. Linear regression data for

calibration curve was shown in fig. 3. The range of solution has been decided according to a correlation coefficient of the regression equation.

Precision

Repeatability study was carried out with six replicates, and intermediate precision studies were carried out with three concentrations of midodrine hydrochloride with three replicates. The values of % relative standard deviation (% RSD) of precision study are shown in table 3.

Robustness

Robustness of the optimized method was studied by changing flow rate (± 0.1 ml/min), change in wavelength (± 2 nm) and change in mobile phase composition ($\pm 5\%$) during analysis. The sample was injected in triplicate for every condition and % RSD was calculated for each condition is shown in table 5.

Limit of detection (LOD) and limit of quantitation (LOQ)

Five sets of concentrations were prepared between 19.98-99.90 $\mu\text{g/ml}$ and the corresponding areas of these sets were measured. Calibration curves were plotted for each set. The standard deviation of the y-intercept and average slope of the calibration curve was used to calculate LOD and LOQ using following formulae.

$$LOD = \frac{3.3 \times SD}{S} \quad LOQ = \frac{10 \times SD}{S}$$

Where SD is the standard deviation of y-intercepts of the calibration curves; S is the mean slope of six calibration curves.

Accuracy

The accuracy of the method was determined by calculating percent recovery of the drug by standard addition method. Percent recovery of midodrine hydrochloride was determined at three different level 80%, 100%, and 120% of the target concentration in triplicate. The results of accuracy study are shown in table 4.

Forced degradation studies

To evaluate stability, midodrine hydrochloride was subjected to force degradation under the condition of acid, base, neutral hydrolysis and oxidation as per international conference on harmonization (ICH) guidelines [15].

Alkaline hydrolysis

100 mg of midodrine hydrochloride was weighed accurately and transferred in 250 ml round bottom flask containing 100 ml of 0.1N sodium hydroxide (NaOH). This mixture was refluxed at 80 °C. After every 30 min 5 ml of refluxed sample was withdrawn and neutralized with 5 ml of 0.1 N hydrochloric acid. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 $\mu\text{g/ml}$. The chromatogram obtained after 2 h of alkali hydrolysis is shown in fig. 4A.

Acid hydrolysis

100 mg of midodrine hydrochloride was weighed accurately and transferred in 250 ml round bottom flask containing 100 ml of 0.1N hydrochloric acid (HCl). This mixture was refluxed at 80 °C. After every 30 min 5 ml of refluxed sample was withdrawn and neutralized with 5 ml of 0.1 N sodium hydroxide. This solution was

further diluted 10 times with mobile phase to obtain a concentration of 100 $\mu\text{g/ml}$. The chromatogram obtained after 2 h of acid hydrolysis is shown in fig. 4B.

Oxidative degradation

100 mg of midodrine hydrochloride was weighed accurately and transferred in 250 ml round bottom flask containing 100 ml of 30% hydrogen peroxide (H_2O_2). This mixture was refluxed at 80 °C. After 30 min 5 ml of refluxed sample was withdrawn. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 $\mu\text{g/ml}$. The chromatogram obtained after 30 min of oxidative degradation is shown in fig. 4C.

Hydrolytic degradation

100 mg of midodrine hydrochloride was weighed accurately and transferred in 250 ml round bottom flask containing 100 ml water (H_2O). This mixture was refluxed at 80 °C. After every 30 min 5 ml of refluxed sample was withdrawn. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 $\mu\text{g/ml}$. The chromatogram obtained after 2 h of hydrolytic degradation is shown in fig. 4D.

RESULTS AND DISCUSSION

Selection of detection wavelength

Detection wavelength of midodrine hydrochloride was selected as wavelength maxima (λ_{max}) from UV absorption spectrum of the drug between 200-400 nm. The value of λ_{max} was found to be 289 nm.

Optimization of chromatographic conditions

Initially, various chromatographic conditions were tried in order to obtain better separation characteristics by changing mobile phase composition and pH. Finally, mobile phase triethylamine buffer 0.02% v/v (pH-3): acetonitrile (38:62 v/v) at the flow rate 0.6 ml/min was selected with UV detection at 289 nm. The retention time of midodrine hydrochloride was found to be 3.56 min. indicated that the method is rapid. The chromatogram of midodrine hydrochloride is shown in fig. 2 and optimized chromatographical conditions are mentioned in table 1.

Table 1: Optimized chromatographic conditions

Parameters	Details
Mobile phase	Triethylamine buffer 0.02% (pH-3): acetonitrile (38:62 v/v)
Column	'Enable' C ₁₈ , 250 mm X 4.6 mm, 5 μm
Flow rate	0.6 ml/min
Detection	UV at 289 nm
Injection volume	20 μl
Runtime	15 min
Retention time	3.564 \pm 0.02 min.
Diluent	Triethylamine buffer 0.02% (pH-3): acetonitrile (38:62 v/v)

Assay of tablet formulation

The value of mean % drug found to be 98.70% which was within acceptance criteria.

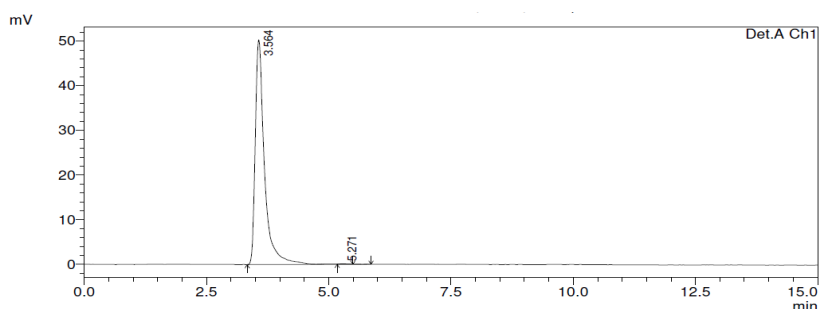


Fig. 2: Chromatogram of midodrine hydrochloride

Table 2: Results of assay of midodrine hydrochloride

S. No.	Sample solution concentration ($\mu\text{g/ml}$)	Actual concentration found	Amount of drug estimated mean \pm SD*
1	60	59.04	98.70 \pm 0.219
2	60	59.17	
3	60	59.47	

*The value is represented as a mean \pm SD of 3 observations.

Table 3: Repeatability and intermediate precision for midodrine hydrochloride

Precision	Concentration of drug ($\mu\text{g/ml}$)	Mean area \pm SD*	% RSD
Repeatability (n=6)	59.94	1 793 956 \pm 1901.33	0.105
Intra-day (n=3)	39.96	1 204 837 \pm 5773.62	0.479
	59.94	1 787 905 \pm 5884.4	0.329
Inter-day (n=3)	79.92	2 362 555 \pm 2413.44	0.102
	39.96	1 218 635 \pm 5239.69	0.430
	59.94	1 790 171 \pm 10058.9	0.561
	79.92	2 191 845 \pm 2872.63	0.630

*Each value is represented as a mean \pm SD of n observations. The value of n is 6 for repeatability study and 3 for intraday and interday precision. SD: Standard deviation, %RSD: Percent relative standard deviation

Table 4: Recovery study for midodrine hydrochloride

Levels	Amount taken ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% recovery*	Mean %recovery \pm %RSD
80%	48	46.60	97.08	97.08 \pm 0.006
		46.62	97.09	
		46.59	97.07	
100%	60	58.88	98.14	98.25 \pm 0.032
		59.02	98.37	
		58.95	98.25	
120%	72	71.17	98.85	98.94 \pm 0.091
		71.24	98.95	
		71.31	99.04	

*Percent recovery was done in triplicate, % recovery: Percent recovery, %RSD: Percent relative standard deviation

Linearity and range

The value of correlation coefficient for midodrine hydrochloride (fig. 3) demonstrated the good relationship between peak areas and concentrations. Therefore, the developed method was linear in the concentration range of 19.98-99.90 $\mu\text{g/ml}$.

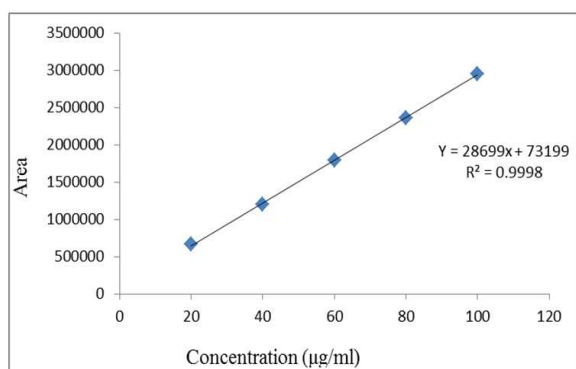


Fig. 3: Calibration curve of midodrine hydrochloride

Precision

The method is precise and the %RSD values were within an acceptable limit.

Accuracy

The value of mean % recovery and % RSD at each level was found within acceptance criteria that indicate the method is accurate.

Robustness

The value of % RSD was found to be within acceptance criteria which showed the reliability of the method.

Table 5: Robustness study for midodrine hydrochloride

Factor	%RSD*
A: Change in flow rate	
0.8 ml/min	0.014
0.9 ml/min	0.023
1.0 ml/min	0.018
B: Change in wavelength	
288 nm	0.030
290 nm	0.421
292 nm	0.397
C: Change in mobile phase composition	
Triethylamine Buffer: Acetonitrile (34:66)	0.068
Triethylamine Buffer: Acetonitrile (36:64)	0.038
Triethylamine Buffer: Acetonitrile (35:65)	0.020

*Each value is represented as % RSD of n observations. The value of n is 3 for change in flow rate, change in wavelength and change in mobile phase composition. %RSD: Percent relative standard deviation

Limit of detection (LOD) and limit of quantitation (LOQ)

The values of limit of detection and limit of quantitation were found to be 2.43 and 7.41 $\mu\text{g/ml}$; respectively that indicated the method is sensitive.

Forced degradation studies

Midodrine hydrochloride was degraded under different stress conditions like alkaline, acidic hydrolysis, oxidative degradation and hydrolytic degradation.

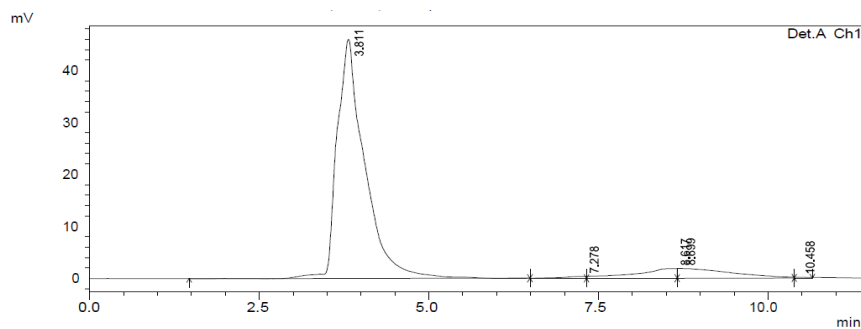
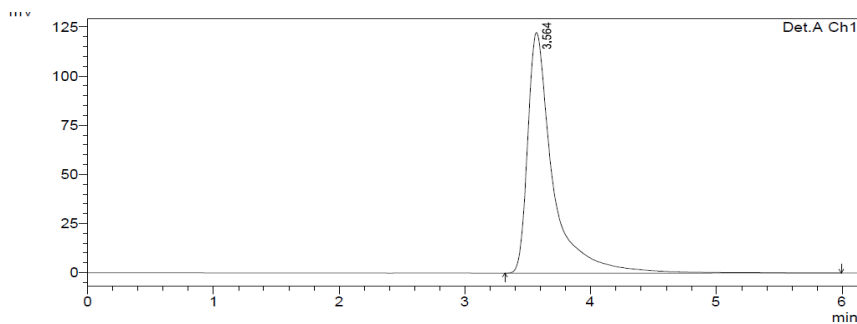
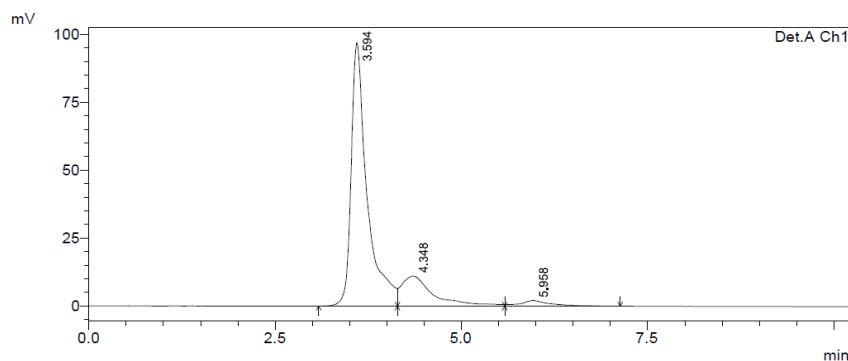
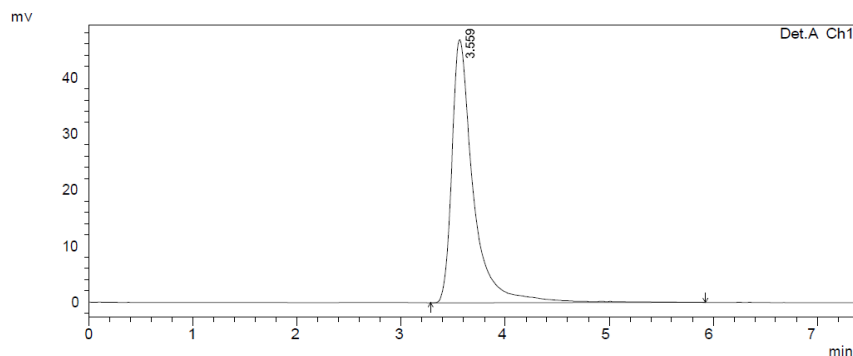
**Fig. 4 (A)****Fig. 4 (B)****Fig. 4(C)****Fig. 4(D)**

Fig. 4: Typically degradation chromatograms of midodrine hydrochloride; (A): in 0.1 N NaOH at 80 °C after 2h. (B): in 0.1 N HCl at 80 °C after 2h. (C): in 30% H₂O₂ at 80 °C after 30 min. (D): in water at 80 °C after 2h

Initially, 0.1 N NaOH solution was used for degradation of midodrine hydrochloride at 80 °C for 30 min but there was no degradation. So same solution was further used at 80 °C for 2h and the chromatogram (fig. 4A) showed that one degraded product was found at a retention time of 8.6 min. Then, 0.1 N HCl solution was used at 80 °C for 2hr and the chromatogram (fig. 4B) indicated that the drug was stable. The other study involved the use of 30% H₂O₂

for 30 min. and the chromatogram (fig. 4C) showed that two degraded products were found at a retention time of 4.34 and 5.95 min. Finally, water was used at 80 °C for 2hr and the chromatogram (fig. 4D) showed that intensity of the peak was decreased due to degradation of the drug. The results of forced degradation studies indicated that the drug was stable in acidic condition and degraded in basic, oxidative and hydrolytic conditions.

The presently developed method represents the first report for stability indicating HPLC assay of this drug and results of the method validation were found within acceptance criteria. However, the existing bioanalytical methods [7, 8] do not have forced degradation studies, utilizing sophisticated instruments and expensive methods.

CONCLUSION

The present study represents a first stability-indicating HPLC assay method that deals with the estimation of midodrine hydrochloride in tablets. The method was successfully validated as per ICH guidelines Q2 (R1). Results of stress testing study revealed that the method is stability indicating. It can be concluded from the results that the developed method is simple, rapid, accurate, specific, sensitive and precise. Thus, this method can be used for routine analysis of midodrine hydrochloride formulation and to check the stability of bulk samples.

ACKNOWLEDGEMENT

Authors thank Principal, Sinhgad College of Pharmacy, Pune for providing required facility to complete this project.

CONFLICT OF INTERESTS

Declared none

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How to cite this article

- Hemant K Jain, Kishor N Gujar, Varsha A Randhe. Stability indicating RP-HPLC assay method for estimation of midodrine hydrochloride in bulk and tablets. *Int J Pharm Pharm Sci* 2016;8(9):283-287.