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# Stability-Testing RP-HPLC Method for Quantifying Midodrine Hydrochloride in Bulk and Oral Solution

**Abhijeet A. Sonawane[1], Saurabh B. Raut[2], Sonal G. Sahane[3], Prapti A. Khemnar [4], Pratibha B. Gunjal[5], Sachin S. Kadam[6], Dushyant D. Gaikwad[7], Suresh L Jadhav[8]**
[1-8]Vishal Institute of Pharmaceutical Education and Research, Ale, Tal. Junnar Dist.- Pune Maharashtra, 412411.

**Email:**

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*Midodrine Hydrochloride, RP-HPLC, Method validation, Stability indicating assay method*

**ABSTRACT**
**The chromatographic separation was performed on GL-Science, Inertsil ODS 3V C18 (5µm, 4.6x 150 mm) column. The mobile phase consists of phosphate buffer pH-4: acetonitrile (95:5 v/v) was delivered at a flow rate of 1 ml/min and UV detection at 290 nm. The method was validated with forced degradation studies as per ICH guidelines. The retention time of the drug was found to be 3.7 min. The developed method was found to be linear in a concentration range of 125-375 µg/ml of the drug ($r^2$=0.999). The low value of % RSD indicates reproducibility of the method. The results of forced degradation studies indicated that the drug was degraded in acidic, basic, oxidative and photolytic conditions**



## INTRODUCTION:

(±)-2-amino-N-(β-hydroxy-2, 5-dimethoxyphenethyl) acetamide monohydrochloride is the chemical formula for midodrine hydrochloride (MD)[1]. Desglymidodrine is an active metabolite of midodrine, a prodrug. As an alpha-1 agonist, desglymidodrine raises blood pressure and vascular tone by activating α-adrenergic receptors in the venous and arteriolar vasculature[2]. The United States Pharmacopeia (USP) officially recognizes this medication. According to a review of the literature, midodrine hydrochloride can be determined using spectrophotometric techniques[3, 4], ion selective techniques[5], and HPLC [6]. Additionally, midodrine hydrochloride was detected in plasma using the LC-MS/MS method[8] and the HPLC approach with fluorescence detection[7]. As of yet, no stability-indicating HPLC assay technique for estimating midodrine hydrochloride in pharmaceutical formulations and bulk has been published. Therefore, the present work involves the development of a stability indicating RP-HPLC assay method for estimation of this drug in bulk and oral solution.

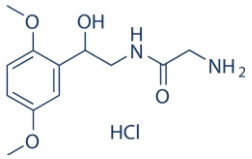


**Fig. 1: Chemical structure of midodrine hydrochloride.**

## MATERIALS AND METHODS:

### Chemicals and Reagents:

Midodrine hydrochloride was purchased from industry Enaltec pharma research pvt ltd Mumbai Commercially available oral solution (Midodrine HCl oral solution®: 0.5 mg/ml of midodrine hydrochloride) used in this study were purchased from Enaltec pharma research pvt ltd Mumbai. HPLC grade of solvents was procured from Enaltec pharma research Pvt. Ltd., Mumbai and AR grade of chemicals were obtained from Merck Pvt. Ltd., Mumbai.

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**Instruments:**
The method was performed on Waters (Japan) Gradient System liquid chromatography solvent delivery system Reciprocating pump Waters- 510, Waters 2695 PDA, Empower Pro data station was used as a data processor. The chromatographic separation was carried out on GL-Science, Inertsil ODS 3V C18, 5µ, 4.6 X 150mm column. Digital Analytical balance (Mettler Toledo) were used for this work.

**EXPERIMENTAL 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 diluent. Filter the sample solution through 0.45µ membrane PVDF filter, discard first 2mL filtrate. (Concentration of Midodrine Hydrochloride standard solution: (0.25mg/mL).

**Preparation of Midodrine HCL oral Solution:**
Transfer 5 mL of Midodrine HCL oral solution in 10 mL volumetric flask, add 3 ml diluent, mix well and dilute up to volume with diluent. Filter the sample solution through 0.45µ membrane PVDF filter, discard first 2mL filtrate. (Concentration of Midodrine Hydrochloride sample solution: 0.25mg/mL)

**Validation of the Method:**
The developed chromatographic method was validated for system suitability, linearity, range, accuracy, precision Specificity, robustness and Forced Degradation Study parameters, as per ICH guidelines[9-12].

**1. System Suitability:**
System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from Single injection of blank (Diluent) and five replicate injections of Standard solution were injected into the chromatograph. The data obtained is summarized in Table no 2.

**2. Linearity:**
Linearity was evaluated in the range of 50 % to 150 % of Midodrine Hydrochloride for working concentration. The working concentration of Midodrine Hydrochloride in Midodrine Hydrochloride is 0.25mg/mL. The data summarized in Table no 3.

**3. Accuracy (Recovery):**
Evaluated accuracy from 50% to 150% of Midodrine Hydrochloride Oral solution, 0.5mg/mL working concentration level. The working concentration of Midodrine Hydrochloride in Midodrine Hydrochloride Oral solution, 0.5mg/mL is 0.25mg/mL, each level prepared in triplicates. The data obtained is summarized in Table no 4.

**4. Precision:**
**4.1 System Precision:**
Single injection of Blank (Diluent) and five replicate injections of Standard solution were injected into the chromatographic system. The data obtained is summarized in Table no 5.

**4.2 Method Precision:**
Single injection of blank (Diluent), Standard solution (five replicates) and sample solution (six preparations) was injected on the system. For system suitability parameter refer the data from Table no 1. The data obtained is summarized in table no 6.

**4.3 Intermediate Precision:**
Five independent sample preparations were prepared on different day and by different analyst and injected on the HPLC. The data obtained is summarized in table no 6.

**5. Specificity: (Identification, Interference & Peak Purity)**
Inject Blank (Diluent), standard solution, impurity Solution, placebo solution and sample solution. The data obtained is summarized in Table no 7.

**5.1 Robustness:**
This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions. The data obtained are summarized in Table no 8.

6 **Forced Degradation Studies:**
To evaluate stability, midodrine hydrochloride was subjected to force degradation under the condition of acid, Alkali, Oxidative, Photo Degradation as per international conference on harmonization (ICH) guidelines [13].

**6.1 Acid Degradation:**
**Sample preparation:** Transferred 5 mL of Midodrine HCL sample solution in 10 mL volumetric flask. Added 2 mL of 2 N Hydrochloric acid solution and kept it for 24 Hrs. in room temperature after exposed added 2 mL of 2 N Sodium hydroxide solution to neutralize and mixed well. Diluted to volume with diluent mixed well and filter the sample solution through 0.45µ membrane

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PVDF filter, discard first 2 mL filtrate. Acid degradation shown in fig.2.

### 6.2 Alkali Degradation:

**Sample preparation:** Transferred 5 mL Midodrine HCL sample solution in to 10 mL volumetric flask. Added 1.5 mL of 1N Sodium hydroxide solution in it and kept it 6 Hrs at room temperature. After exposed added 1.5 mL 1 N hydrochloric acid solution mix and diluted to volume with diluent. Mix well and filter the sample solution through 0.45µ membrane PVDF filter, discard first 2 mL filtrate. Alkali degradation shown in fig.3.

### 6.3 Oxidative Degradation:

**Sample preparation:** Transferred 5 mL of Midodrine HCL sample solution in to 10 mL volumetric flask. Added 3 mL of 50% Hydrogen peroxide and kept it into water bath for 6 Hrs at 80ºC. After exposed allow it to cool at room temperature and diluted to volume with diluent. Mix well and filter the sample solution through 0.45µ membrane PVDF filter, discard first 2 mL filtrate. Oxidative degradation shown in fig.4.

### 6.4 Photo Degradation: Sample preparation:

Transferred 5 mL of sample solution separately in two different 10 mL volumetric flask **(**One as such exposed and another wrapped with aluminum foil) and exposed it under UV and white light for 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt/square. After exposure, added 3 mL of diluent mixed well and dilute up to volume with diluent. Filter the sample solution through 0.45µ membrane PVDF filter, discard first 2 mL filtrate. Photo degradation shown in fig.5.

## RESULTS AND DISCUSSION:

### Selection of detection wavelength:

Detection wavelength of midodrine hydrochloride was selected as wavelength maxima (λmax) from UV absorption spectrum of the drug between 200-400 nm. The value of λmax was found to be 290 nm.

### Optimized Chromatographic Conditions:

**Table 1: Optimized Chromatographic Conditions:**

| Chromatographic Conditions | Values |
|---|---|
| Elution | Isocratic |
| Mobile phase | phosphate buffer PH-4 and Acetonitrile |
| Mobile phase concentration | 95:5 v/v |
| Column | Inertsil ODS 3V C18, 5µ, 4.6 x 150mm |
| Flow rate | 1.0 mL/min |
| Injection volume | 20 µL |
| Wavelength | 290 nm |
| Sample temp | 10ºC |
| Run time | 10.0 minutes |

**Table 2: Result for system suitability test of Midodrine Hydrochoride.**

| **Component** | **Midodrine Hydrochloride** |
|---|---|
| **USP Tailing** | 1.2 |
| **Theoretical Plates** | 3481 |
| **S. No.** | Area |
| 1 | 3373450 |
| 2 | 3375982 |
| 3 | 3376214 |
| 4 | 3378952 |
| 5 | 3375142 |
| **Mean Area** | 3375948 |
| **%RSD** | 0.059 |
| **Correlation** | 99.8 |

**Table 3. Linearity of Midodrine Hydrochoride**

| **Level (%)** | **Concentration Vs Sample(ppm)** | **Area** |
|---|---|---|
| 50% | 124.9 | 1682399 |
| 75% | 187.3 | 2506349 |
| 100% | 249.7 | 3332293 |
| 125% | 312.2 | 4175532 |
| 150% | 374.6 | 5007562 |
| **Correlation Co-coefficient(r)** | | **1.000** |
| **Slope of regression line** | | **13324.82** |
| **%Y- intercept** | | **0.40** |
| **Regression ($r^2$)** | | **0.999** |

**Table 4: % Recovery for Midodrine Hydrochloride**

| **Level %** | **Mean Response** | **% Recovery** | **% Mean recovery** |
|---|---|---|---|
| 50 | 1675479.00 | 99.9 | 100.0 |
| | 1677434.00 | 100.0 | |
| | 1678154.00 | 100.1 | |
| 100 | 3334222.00 | 99.4 | 99.5 |
| | 3336251.00 | 99.5 | |
| | 3339567.00 | 99.6 | |
| 150 | 5021070.00 | 99.8 | 99.8 |
| | 5020536.00 | 99.8 | |
| | 5017280.00 | 99.7 | |

**Table 5: System precision**

| **Component** | **Midodrine Hydrochloride** |
|---|---|
| **USP Tailing** | 1.2 |
| **Theoretical Plates** | 3481 |
| **S. No.** | Area |
| 1 | 3373450 |
| 2 | 3375982 |
| 3 | 3376214 |
| 4 | 3378952 |
| 5 | 3375142 |
| **Mean** | 3375948 |
| **%RSD** | 0.059 |
| **Correlation** | 99.8 |

**Table 6: Intermediate Precision.**

| **Parameter** | **Method Precision** | **Intermediate Precision** |
|---|---|---|

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| | (Analyst-I) | (Analyst-II) |
|---|---|---|
| **HPLC NO.** | EPR/AD/I-022 | EPR/AD/I-053 |
| **Column No.** | C18-611 | C18-298 |
| **Sample No.** | %Assay | |
| 1 | 97.1 | 97.0 |
| 2 | 97.2 | 97.0 |
| 3 | 97.2 | 97.0 |
| 4 | 97.0 | 97.0 |
| 5 | 97.1 | 97.0 |
| 6 | 97.2 | 97.0 |
| **Mean** | 97.1 | 97.0 |
| **Absolute Mean difference % assay** | 0.1 | |

**Table 7: Specificity (Identification and Interference)**

| Solution | Specificity data | | |
|---|---|---|---|
| | Retention time (min) | Purity Match | |
| Blank solution | NA | NA | |
| Placebo solution | NA | NA | |
| Standard solution | 3.74 | Purity angle | Purity threshold |
| | | 0.083 | 0.269 |
| Sample solution | 3.73 | 0.061 | 0.283 |
| Midodrine Impurity A | 3.36 | 0.320 | 0.597 |

**Table 8: System Suitability for Robustness**

| Changes in parameters | Values | A | B | C | D | E |
|---|---|---|---|---|---|---|
| Control | As per method | 3.74 | 1.2 | 3481 | 0.12 | 99.8 |
| Change in Flow rate 1.0 mL/min (±0.1 mL/min) | 0.9 mL/min | 4.08 | 1.2 | 3626 | 0.04 | 99.7 |
| | 1.1 mL/min | 3.39 | 1.2 | 3131 | 0.10 | 99.7 |
| Change in Wavelength (±2 nm) | 288 nm | 3.74 | 1.2 | 3481 | 0.13 | 99.8 |
| | 292 nm | 3.74 | 1.2 | 3481 | 0.13 | 99.8 |
| Change in Column temperature (±5 °C) | 20°C | 3.86 | 1.2 | 3302 | 0.08 | 99.8 |
| | 30°C | 3.51 | 1.2 | 3369 | 0.09 | 99.9 |
| Change in pH of buffer for Mobile Phase (± 0.2 unit) | pH 4.0 | 3.86 | 2.0 | 7115 | 0.18 | 100.0 |
| | pH 3.8 | 3.92 | 2.0 | 6694 | 0.09 | 100.0 |
| | pH 4.2 | 3.88 | 1.9 | 6384 | 0.07 | 99.6 |

## Forced Degradation Studies:

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

**Table 9: Force Degradation for Midodrine Hydrochoride**

| Reagents | Conditions | % Assay | Purity angle | Purity threshold |
|---|---|---|---|---|
| Acid | 2 mL, 2 N HCl for 24 Hrs | 92.3 | 0.010 | 0.236 |
| Base | 1.5 mL, 1 N NaOH for 6 Hrs | 87.7 | 0.175 | 0.235 |
| Peroxide | 3 mL H2O2 and heat at 80 ºC for 6 Hrs | 90.3 | 0.069 | 0.466 |
| Photo-Open | 1.2 million lux Hrs and 200 watt/square | 95.6 | 0.029 | 0.255 |
| Photo-Close | 1.2 million lux Hrs and 200 watt/square | 97.2 | 0.023 | 0.259 |

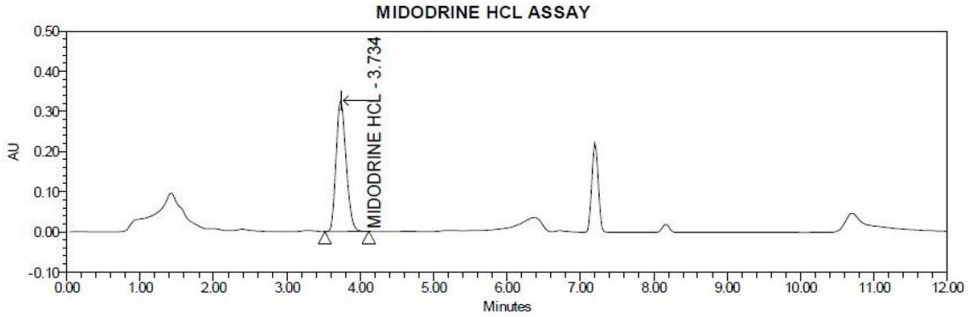


**Fig no 2. Acidic Degradation of Midodrine Hydrochloride Oral Solution**

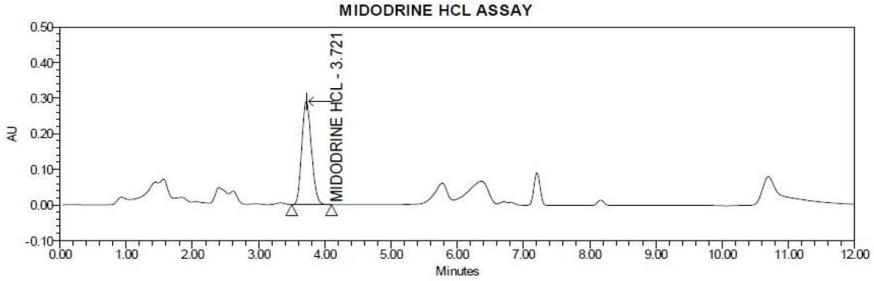


**Fig no 3: Alkaline Degradation of Midodrine Hydrochloride Oral Solution**

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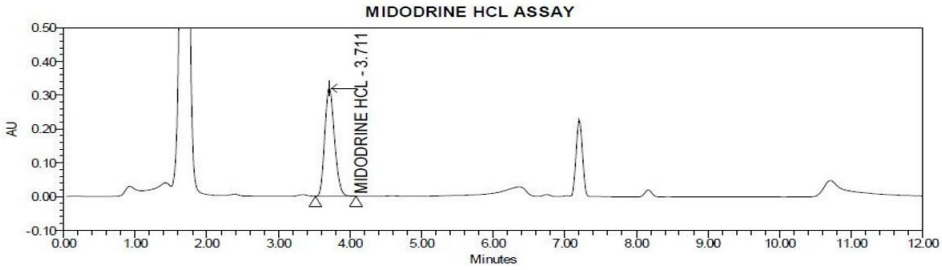


**Fig no 4: Oxidative Degradation of Midodrine Hydrochloride Oral Solution**

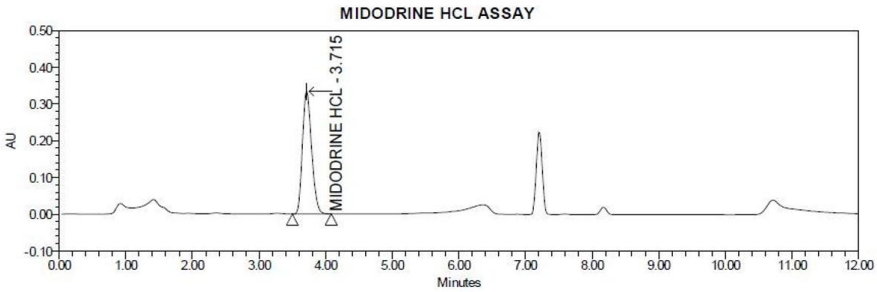


**Fig no 5: Photo Degradation of Midodrine Hydrochloride Oral Solution (open)**

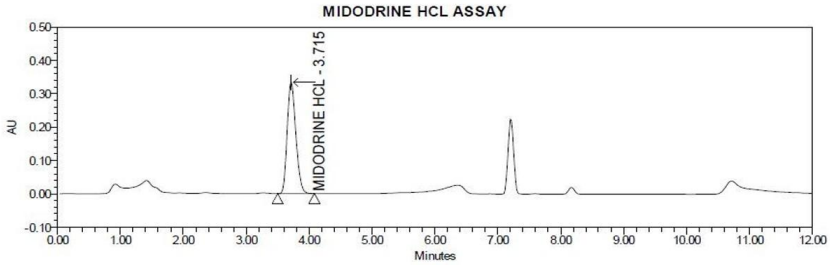


**Fig no 6: Photo Degradation of Midodrine Hydrochloride Oral Solution (close)**

**Results**: The retention time of the drug was found to be 3.7 min. The developed method was found to be linear in a concentration range of 125-375 µg/ml of the drug ($r^2$=0.999). The low value of % RSD indicates reproducibility of the method. The results of forced degradation studies indicated that the drug was degraded in acidic, basic, oxidative and photolytic conditions.

## CONCLUSION:

The current study introduces a novel, stability-indicating RP-HPLC assay method for the quantification of midodrine hydrochloride in oral solution. The method underwent successful validation in accordance with ICH guidelines Q2(R1). Stress testing results confirmed the method's ability to indicate stability. Based on these findings, it can be concluded that the developed method is straightforward, rapid, accurate, specific, sensitive, and precise. Therefore, this method is deemed suitable for routine quality control analysis of midodrine hydrochloride oral solution and is valid for its intended purpose.

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