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STABILITY-INDICATING HPTLC METHOD FOR DETERMINATION OF MIDODRINE HYDROCHLORIDE

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ABSTRACT

A simple and rapid stability indicating HPTLC method for Midodrine Hydrochloride was successfully developed. This method is based on HPTLC separation followed by UV detection at 290 nm. The separation was carried out on merck TLC aluminium sheets precoated with silica gel $60F_{254}$ using n-butanol: Methanol: water (6:2:2 v/v/v) as a mobile phase. Midodrine Hydrochloride gave well defined and sharp peak at R_f 0.30 ± 0.02. Calibration curve was linear in range 400-1200 ng/band. Stress degradation study shows that sample degraded with acid and base hydrolysis, under oxidation, thermal and photolytic stress conditions. The peak purity parameter ensured noninterference by product of degradation. This method can be applied to determination of stability of Midodrine HCl. The suitability of this HPTLC method for quantitative determination of Midodrine Hydrochloridewas proved by validation in accordance with requirements of ICH guidelines.

KEYWORDS: Midodrine Hydrochloride, HPTLC, Forced degradation, Validation.

INTRODUCTION

Midodrine Hydrochloride is used as Vasopressor or Antihypotensive. Chemically it is 2-amino-N-[2-(2,5-dimethoxyphenyl) -2- hydroxyethyl] acetamide; hydrochlorid. Midodrine hydrochloride forms an active metabolite, desglymidodrine, that is an alpha1-agonist, and exerts its actions via activation of the alpha-adrenergic receptors of the arteriolar and venous vasculature, producing an increase in vascular tone and elevation of blood pressure.[1]

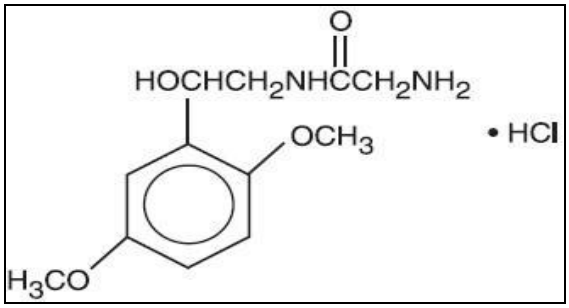

Fig.1: Chemical structure of Midodrine HCl.

A thorough literature search indicated that there are some methods reported for estimation of Midodrine Hydrochloride by Electrochemical methods[2], HPLC[3-4], LC-MS/MS[5] , methods. To the best of our knowledge there is no Stability-Indicating HPTLC Method (SIM) reported for Midodrine Hydrochloride. Hence, considering inherent advantage of HPTLC over HPLC, the objective of current work was to develop Stability-Indicating HPTLC Method (SIM) as per ICH Q1A (R2) guidelines. It was aimed to establish inherent stability of the Midodrine Hydrochloride through stress studies under a variety of stress conditions and to validate the Stability-Indicating Assay method.

MATERIALS AND METHODS

Chemicals and reagents

Working standard of Midodrine Hydrochloride was kindly supplied by Cadila Healthcare Limited, Vadodara, Gujarat, India. n-butanol and methanol (AR grade) were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Precise analytical weighing balance (Shimadzu AY120) was used for weighing. Chromatographic separation of drug was performed using aluminium plate precoated with silica gel 60 F_{254} (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Thermal degradation study was carried out in hot air oven (Make - Kumar lab).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using n-butanol: methanol: water (6:2:2 v/v/v) as mobile. The optimized chamber saturation time for mobile phase was 15 min. The length of

chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 290 nm operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Stock Solution

Standard stock solution of Midodrine Hydrochloride was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. from which 1 ml was further diluted to 10 ml with methanol to get concentration of solution 100 µg/ml

Selection of Detection Wavelength

The UV spectrum of Midodrine Hydrochloride (10 µg/ml) solution was obtained over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 290 nm. So, wavelength 290 nm was selected as the wavelength for detection.

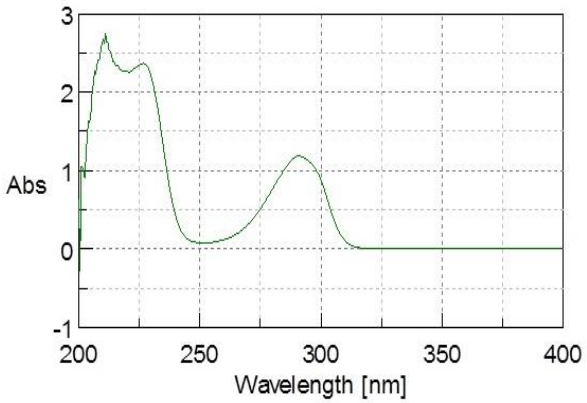

Fig.2: UV Spectrum of Midodrine Hydrochloride (10µg/ml)

Stress degradation studies of bulk drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under acid, base and neutral hydrolytic, oxidative, thermolytic and photolytic conditions. Stress conditions were optimized to achieve 10 to 30 % degradation

Acid treatment

1 ml working standard solution of Midodrine Hydrochloride (1000 µg/ml) was mixed with 1 ml of 0.01N hydrochloric acid (HCl) and 8 ml of methanol. Solution was kept at room temperature for 1 hour. The 8 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Alkali treatment

1 ml working standard solution of Midodrine Hydrochloride (1000 µg/ml) was mixed with 1 ml of 0.1 N sodium hydroxide (NaOH) and 8 ml of methanol. Solution was kept at room temperature for 24 hour. The 8 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Neutral Hydrolysis

1 ml working standard solution of Midodrine Hydrochloride (1000 µg/ml) was mixed with 1 ml of water and 8 ml of methanol. Solution was kept at room temperature for 24 hour. The 8 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Oxidative degradation

1 ml working standard solution of Midodrine Hydrochloride (1000 µg/ml) was mixed with 1 ml of 3% v/v Hydrogen peroxide (H_2O_2) and 8 ml of methanol. Solution was kept at room temperature for 4 hour. The 8 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Degradation under dry heat

Dry heat study was performed by keeping drug in oven at 60ºC for period of 12 hours. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 100 µg/ml. 8 µl of the resulting solution was applied to HPTLC.

Degradation under Photolytic conditions

Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt hours /square meter and subsequently to fluorescence light illumination not less than 1.2 million lux hours. Sample was weighed, dissolved in methanol to get concentration of 100 µg/ml. 8 µl of the resulting solution was applied to HPTLC.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary objective in developing this stability indicating HPTLC method is to achieve the resolution of Midodrine Hydrochloride and its degradation products. Representative densitogram of standard solution of Midodrine Hydrochloride is shown in figure: 3.

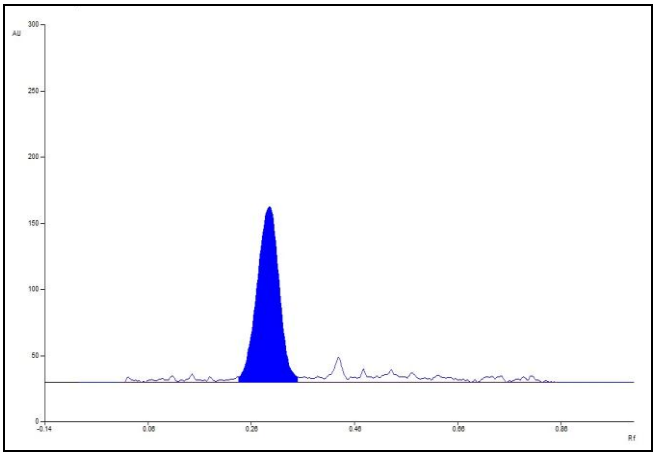

Fig. 3: Representative densitogram of standard solution of Midodrine Hydrochloride (800ng/band, R_f = 0.30 ± 0.02)

Results of forced degradation studies
Forced degradation study showed that the method is highly specific and there was no interference of degradation products observed at retention factor of drug.

Acid treatment
In Acid hydrolysis condition, 72.15% degradation of Midodrine Hydrochloride was observed with no peak of degradation.

Alkali treatment
In alkali hydrolysis condition, 91.61 % degradation of Midodrine Hydrochloride was observed with no peak of degradation.

Neutral Hydrolysis
In neutral hydrolysis condition, 87.01 % degradation of Midodrine Hydrochloride was observed with peak of degradation.

Oxidative degradation
Midodrine Hydrochloride when treated with 3% v/v H_2O_2 i.e. oxidative degradation, 90.43 % degradation was observed.

Dry heat degradation studies
When the drug substance was exposed to dry heat at 60º C for 12 hrs.92.73 % of degradation was observed.

Photo degradation Studies
Midodrine Hydrochloride exhibited 91.51 % of degradation, when exposed to ultraviolet light (200 Watt hours/Sequre meter) and 89.39 % of degradation when exposed to fluorescence light (1.2 million lux hours).

Multiwavelength scanning
All stress conditions of Midodrine Hydeochloride (800 ng/band) and 10 times stress conditions sample (8000 ng/band) were applied on TLC plate. TLC plate was scanned at multiwavelength from 200 nm to 400 nm. There was no degradation product found even when 10 times higher volume was applied.(fig.4)

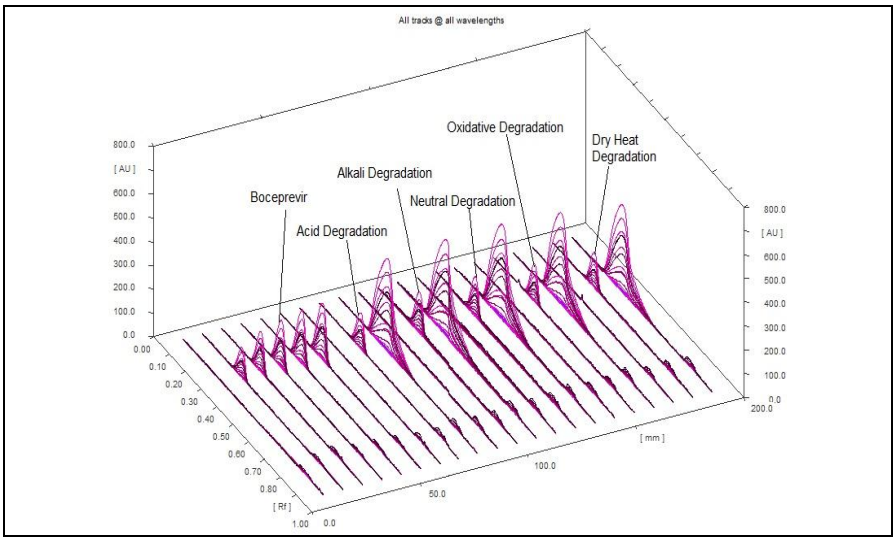

Fig. 4: 3D representative densitogram of all stress conditions scanned at multiwavelength (200nm – 400nm).

The forced degradation studies data is summarized in Table 1.

Table 1: Data of forced degradation studies of Midodrine Hydrochlorid

Stress conditions/ duration	% Recovery	% Degradation
Acidic hydrolysis 0.01 N HCl kept at room temperature for 1 Hr.	72.15	27.85
Alkaline hydrolysis 0.1 N NaOH kept at room temperature for 24 Hr.	91.61	8.39
Oxidative 3 % v/v H_2O_2 /kept at room temperature for 4Hr.	90.43	9.57
Neutral hydrolysis H_2O at room temperature for 24 Hr.	87.01	12.99
Dry heat/ 60ºC/ 12 hours	92.73	7.27
UV light (200 Watt hours/Sequre meter)	91.51	8.49
Fluorescence light (1.2 million lux hours).	89.39	10.61

Validation of the method

The method was validated for various parameter in accordance with ICH guidelines.[8]

1. Specificity

The developed method was specific for analyte. The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.9991, indicating the no interference of any other peak of degradation product.

2. Assay

Marketed sample was not available therefore spiked blend was used. Accurately weighed quantity of Midodrine Hydrochloride was mixed with blank blend (BB) containing starch and lactose. Spiked blend(SB) was assayed and used for accuracy studies. To determine accuracy, 950 mg BB and 50 mg drug were mixed properly by geometric mixing and finally 1000 mg SB were prepared. Blend equivalent to 10 mg of drug was transferred to 10 mL volumetric flask and was diluted with methanol, sonicated for 10 min and volume made to 10 mL (1000 µg/mL). From which 1 ml was further diluted to 10 ml with methanol to get concentration of solution 100 µg/ml. Solution was filtered 4 µl volumes was applied on plate. Analysis was repeated for six times. % assay was determined from linearity equation.

Table 2: Assay of Spiked blend powder.

Sr. No.	Peak area of Midodrine Hydrochloride	Amount Recovered (ng/band)	% Recovery
1	2634.31	402.43	100.60
2	2598.8	395.49	98.87
3	2647.48	405.00	101.25
4	2684.31	412.20	103.05
5	2589.37	393.65	98.41
6	2597.54	395.24	98.81
Mean	2625.30	400.67	100.16
SD	23.28	7.21	1.80
%RSD	0.8915	0.01	0.01

3. Linearity and range

The standard stock solutions of Midodrine Hydrochloride (100µg/ml) were applied by spotting on TLC plate in range of, 4, 6, 8, 10 and 12 µl (Fig.3.9). Straight-line calibration graphs were obtained y = 5.117x + 575.06 in the concentration range 400-1200 ng/band with high correlation coefficient > 0.99. The results obtained are shown in (Table 3), the peak areas were plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig.5 for Midodrine Hydrochloride.

Table 3: Linearity study of Midodrine Hydrochloride.

Replicate	Concentrations of Midodrine Hydrochloride(ng/band)				
	400	600	800	1000	1200
	Peak Area				
1	2591.2	3663.6	4710.5	5687.2	6721.4
2	2599.4	3703.2	4689.5	5717.9	6621.1
3	2599.2	3674.5	4730.5	5617.9	6754.9
4	2587.36	3694.9	4710.5	5717.9	6654.1
5	2499.1	3700	4650.5	5727.2	6694.3
Mean	2575.25	3687.78	4698.3	5693.5	6689.16
Std.dev.	42.88	17.30	30.40	44.86	53.03
%RSD	1.66	0.46	0.64	0.78	0.79

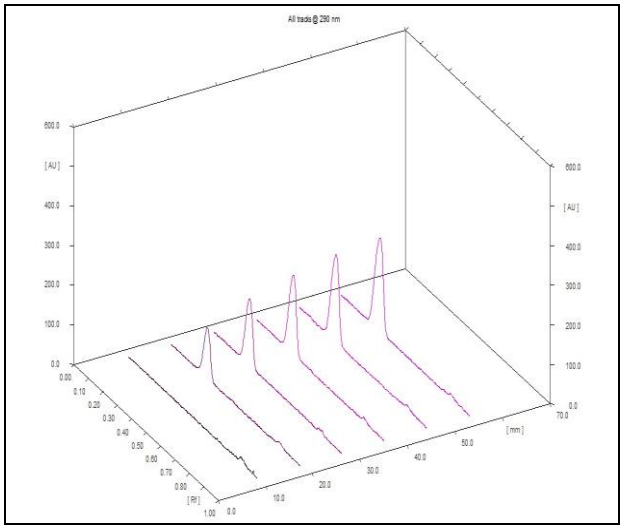

Fig.5: Densitogram of linearity of Midodrine Hydrochloride (400-1200 ng/band)

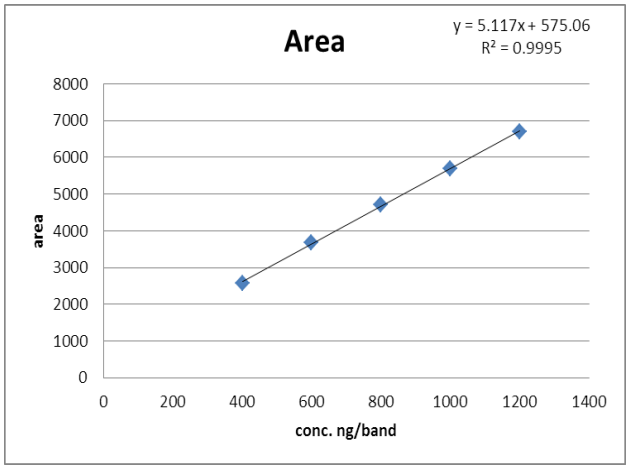

Fig.6: Calibration curve for Midodrine HCl

4. Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample was 4000 ng/band from SB. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate. The results obtained are shown in Table 4.

Table 4: Recovery studies of Midodrine HCl.

Drug	Amount taken (ng/ band)	Amount added (ng/ band)	Total amount found (ng/ band)	% Recovery	% RSD
Midodrine HCl	400	320	728.86	101.23	1.00
	400	400	802.57	100.32	0.50
	400	480	879.65	99.69	1.86

*Average of three determinations

5. Precision

A set of three different concentrations in three replicates of standard solutions of Midodrine Hydrochloride were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.97 to 1.68. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.67 to 0.87. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Table 5: Inter-day precision of Midodrine HCl.

Concentration (ng/band)	Area	Mean Area	SD	% RSD
400	2641.25	2616.5	22.81	0.87
	2596.31			
	2611.94			
800	4650.5	4675.53	31.42	0.67
	4665.3			
	4710.8			
1200	6694.3	6629.45	56.23	0.84
	6594.1			
	6599.95			

Table 6: Intra-day precision of Midodrine HCl.

Concentration (ng/band)	Area	Mean Area	SD	% RSD
400	2591.2	2616.76	36.92	1.41
	2600			
	2559.1			
800	4710.5	4710.16	79.50	1.68
	4639.5			
	4730.5			
1200	6684.25	6675.12	65.23	0.97
	6735.32			
	6605.81			

6. Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response at lowest concentration in range and S is the slope of the calibration plot. The LOD and LOQ were found to be 27.49 ng/band and 83.31 ng/band respectively.

7. Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, chamber saturation time was altered and the effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2). The results are given in Table 7.

Table 7: Robustness Data in Terms of Peak Area (% RSD)

Sr No.	Parameter	(% RSD)
1.	Mobile phase saturation time 10 min and 20 min	0.12
2.	Mobile phase variation n-butanol 5.8 and 6.2 ml	0.25

*Average of three determinations

CONCLUSION

The developed method is stability indicating, since the drug peak was found to be pure as confirmed by peak purity profiling study, under all stress degradation conditions. This proves that there is no interference of degradation product in analytical peak. The method is specific, accurate, precise, and robust and can be used for routine quality control as well as assessing the stability of Midodrine HCl.

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