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Development and Validation of Stability Indicating HPTLC Method for the Estimation of Midodrine HCL in Tablet Dosage Form

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ABSTRACT
This study was to develop and validate a stability indicating high performance thin- layer chromatography (HPTLC) method for the estimation of Midodrine HCl in tablet dosage form. Chromatographic method was developed for estimation of Midodrine HCl in marketed formulation by measuring absorbance at maximum at 280 nm. HPTLC method was developed for estimation of Midodrine HCl by using precoated silica gel aluminium plate 60F-254 (20x20) cm with 250µm thickness 0 (E. Merck) and Methanol : Ethyl acetate (7:3 % v/v) as a mobile phase. The accuracy and reliability of the method was assessed by evaluation of linearity (200-1000 ng/band), precision was found to be less than 2% RSD and accuracy was found to be 101.5-99.19% with correlation coefficient 0.997. The limit of detection (LOD) and the limit of quantification (LOQ) for Midodrine HCl was found to be 5.88 ng/band and 17.2 ng/band respectively, the method has been validated as per ICH guideline. The drug was subjected to stress conditions of acid hydrolysis, alkali hydrolysis, photolytic, thermal degradation. Results found to be linear in the concentration range of 200-1000 ng/band. Method validation parameters lie within its acceptance criteria as per ICH Q2 (R1) guideline so this method are specific, linear, accurate and precise. Hence, they can be successfully used for the routine analysis of Midodrine HCl in pharmaceutical dosage form.

Keywords: High Performance thin- layer Chromatography, Midodrine HCl, Pharmaceutical dosage form, Force Degradation Study, Validation Parameters.

I. INTRODUCTION

Midodrine is indicated for the treatment of symptomatic orthostatic hypotension. Midodrine is chemically 2-amino-N-[2-(2,5-dimethoxyphenyl)-2- hydroxymethyl] acetamide; hydrochloride shown in figure 1. Midodrine was approved in the United States by the Food and Drug Administration (FDA) in 1996 for the treatment of dysautonomia and orthostatic hypotension. In August 2010, FDA proposed withdrawing this approval because the manufacturer, Shireplc, failed to complete required studies after the medicine reached the market. Midodrine undergoes metabolism to form its pharmacologically active metabolite, desglymidodrine. Desglymidodrine acts as an agonist at the alpha1-adrenergic receptors expressed in the arteriolar and venous vasculature. WHO has emphasized the need to ensure the Quality of medicinal plant products by using modern controlled techniques and application of suitable standards. The HPTLC works at the same ideas as TLC which includes the principle of separations adsorption. Pharmaceutical products formulated with multiple drugs, commonly referred to as aggregate merchandise, are supposed to fulfill previously unmet sufferers need by combining the healing consequences of two or more capsules in one product. A pharmaceutical product's capacity to maintain its physical, chemical, microbiological, therapeutic, and toxicological specifications in a certain container or closure system is known as stability.

II. MATERIALS AND METHODS

Midodrine was provided gift sample. Formulations were obtained from Local Market. Methanol, Ethyl acetate (all reagents and chemical used in this study were of analytical grade) and silica gel 60F254 precoated TLC aluminum plates [E-Merck].

Selection of the Mobile Phase

The Mobile phase was prepared by mixing of Methanol: Ethyl acetate in the ratio of 7:3, and the mobile phase was transferred into a twin through chamber covered with lid and allowed to stand for 15min before use.

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Preparation of standard stock solution:

Accurately weighed 100mg of quantity of Midodrine HCl reference standard was transferred into 100 ml volumetric flask and dissolved in methanol and sonicated for about 5min with intermittent shaking and diluted up-to mark with methanol to give a stock solution having strength 1000µg/ml.

Preparation of working Standard solution of Midodrine HCl :

From above stock solution transferred 1 ml into 10ml and mark up to with methanolto give a Standard solution having strength 100µg/ml.

Preparation of Test solution:

Twenty tablets of marketed formulation, (5 mg of Midodrine HCl) were taken and average content was determined. Weight equivalent to 5 mg of Midodrine HCl was weighed and transferred in to 50 ml of volumetric flask, dissolved up to mark with methanol. Solution was sonicated and filtered through Whatmann filter paper.

Selection of Wavelength

The solution was prepared by taking 2 mL of working standard solution of Midodrine HCl and diluted to 10ml with methanol to get 20 µg/mL of Midodrine HCl. The solution was scanned between 200-400 nm. Wavelength was selected from the spectra of Midodrine HCl

Validation of the Method

The HPTLC method was validated for accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity and robustness in accordance with ICH Q2 (R1) guideline.

Linearity

The linearity response was determined by analyzing 5 independent levels of calibration curve, Analysis was performed on pre-coated silica gel aluminum plat 60F-254 (20x10 cm with 0.2 thickness) pre- washed with methanol and then dried for 30minutes at room temperature. From the working standard solution (20µg/ml of Midodrine HCl) aliquots of 10, 20, 30, 40, and 50 µL were spotted on TLC plate under nitrogen stream using Dosage Applicator as 50 to obtain final concentration range 200-1000 ng/band of Midodrine HCl.

Precision

The precision of the proposed method was assessed as repeatability (intra day precision) and intermediate precision (inter day precision), both expressed as %RSD. The repeatability was evaluated by preparing six replicates of the standard solution of Midodrine HCl. The %RSD of Intraday and Interday precision were calculated. The repeatability study of the test sample each day. The intermediate precision was calculated from the result of repeatability study carried out on these three days.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

As per ICH guideline, limit of detection and quantitation of the developed method were calculated from the standard deviation of the response (σ) and slope of the calibration curve (S) of each drug using the formula; Limit of detection=3.3* σ/S and Limit of quantitation=10* σ/S.

Recovery Studies

Accuracy is the closeness of the test results obtained by the method to the true value. The accuracy of the method was determined by calculating recoveries of Midodrine by the standard addition method. Accuracy is performed at three levels 80,100,120%.

Preparation of samples for forced degradation study

Preparation for Acid hydrolysis (0.1 N HCl at 60°C)

Force degradation in acidic media was performed by taking 1 mL of Stock Solution of Midodrine HCl to 10 mL volumetric flask. Add 1mL of 0.01N HCL in volumetric flask and kept 60°c for 3 hrs. Then neutralized with 0.1 N NAOH and diluted up to the mark with mobile phase. Solution strength of 100µg/mL

Preparation for Alkali hydrolysis (0.1 N NaOH at 60°C)

Force degradation in acidic media was performed by taking 1mL of Stock Solution of Midodrine HCl to 10 mL volumetric flask. Add 1mL of 0.1 N NaOH in volumetric flask and kept 60°c for 3 hrs. Then neutralized with 1N HCl and diluted up to the mark with mobile phase. The Solution has strength of 100µg/mL

Preparation for hydrogen peroxide

Force degradation study was performed by taking 1mL of Stock Solution of transferred to their respective 10 mL volumetric flask. Add 1mL of 3% H_2O_2 in each volumetric flask and kept at room temperature for 3 hrs. and diluted up to the mark with mobile phase. The Solution has strength of 100 ug/mL

Preparation for thermal degradation

The Thermal degradation study was performing by keeping drug sample in the oven (100 °C) for a period of 24 hrs. The sample was withdrawn, dissolved in methanol and diluted to

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get 100µg/ml. 10ml of the resultant solution was then applied at TLC and Densitogram was developed,

Preparation for photolytic degradation

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/$m^2$for 7 days at 254nm. After exposure, the sample was withdrawn, dissolved in methanol and diluted to get 100µg/ml. 10ml of the resultant solution was then applied at TLC and Densitogram was developed.

III. RESULT AND DISCUSSION

Resolution is the most important criteria for the method, it is imperative to achieve good resolution among the compounds. As per the value of pKa, Log p and solubility of compound various composition of mobile phase were tried. The mobile phase Methanol: Ethyl acetate (7:3) has sharp spot with good resolution. From overlain spectrum both spots show maximum considerable absorbance between 200-360nm. So, wavelength selected for detection was 280nm. Linear correlation was obtained between peak area and concentration in the range 200-1000 ng/spot for Midodrine HCl shown in figure 1.

Calibration curve

The development of HPTLC method for estimation of Midodrine HCl showed a good correlation coefficient (r2= 0.997) in concentration range of 200-1000 ng/spot with the respect to the peak area. The mean value (± SD) of slope and intercept were 1.453 and 673.3, respectively Midodrine HCl shown in figure 2.

Method Validation

The % RSD of Repeatability, Intra-Day and Inter-Day Precision was found to be 0.12%, 0.43-0.39, and 0.49-0.64 for Midodrine HCl shown in Table 1. Limit of detection (LOD) and limit of quantification (LOQ) are described as shown in Table 2. The calibration curve in this study was plotted between the amounts of analyte versus the average response (peak area). The formulation was analyzed and found to contain 4.97 mg of Midodrine HCl in a tablet Table 3. In recovery studies, the analyzed samples were spiked with an extra 80,100, and 120% of standard Midodrine HCl was reanalyzed by the proposed method shown in Table 4. The experiment was conducted in triplicate. This was done to check for the recovery of drugs at different levels in formulation. The peak purity of Midodrine HCl was assessed by comparing the spectra at peak start, peak apex and peak end positions of spot. Optimized degradation conditions for Midodrine HCl which are shown in table. From degradation study it was found that Midodrine HCl was stable in case of oxidative, thermal and photolytic condition while significant degradation was found in case of acid and alkali degradation as shown in Table 5.

IV. CONCLUSION

The developed and validated HPTLC method for the estimation of Midodrine HCl in pharmaceutical dosage form has been found to be simple, accurate, and precise. Hence method can be successfully for the routine analysis of Midodrine HCl in pharmaceutical dosage form. A stability indicating HPTLC method has been developed and validated for estimation of Midodrine HCl in pharmaceutical dosage form. Midodrine HCl degraded significantly under alkaline and oxidative conditions moderately in acidic and comparatively stable in thermal and photolytic conditions. It gives symmetric peak shape and good resolution for Midodrine HCl. Hence, the method can be successfully used for the estimation of Midodrine HCl in pharmaceutical dosage form in regular quality control testing and stability studies.

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Figure:

Figure: 1 Structure of Midodrine HCl

Figure 2: Overlay Chromatogram of Midodrine HCl standard

Figure 3: Chromatogram of Midodrine HCl 400ng/spot and standardwith RF of 0.44

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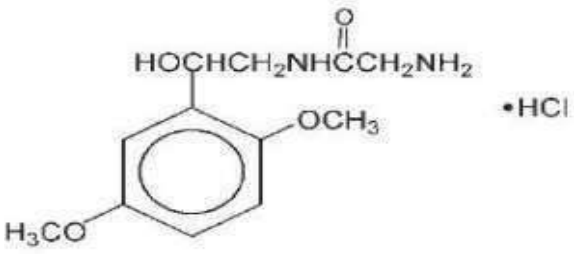

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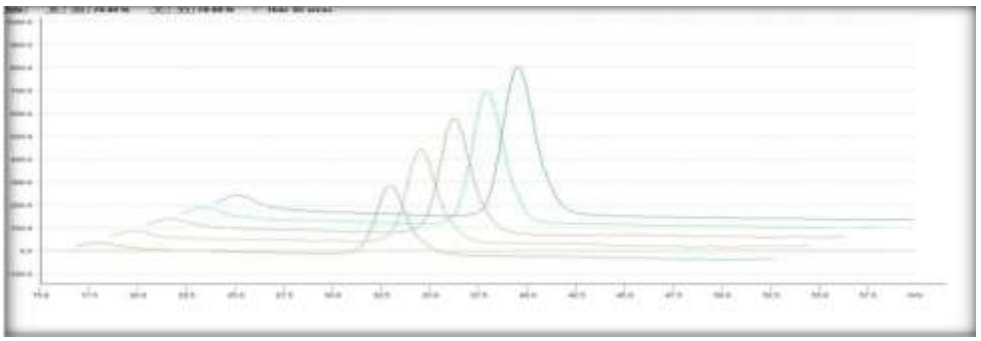

Figure 2: Overlay Chromatogram of Midodrine HCl standard

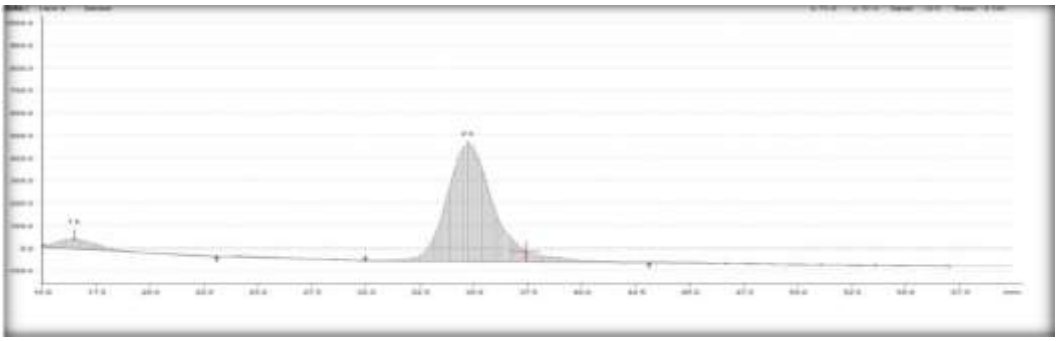

Figure 3: Chromatogram of Midodrine HCl 400ng/spot and standardwith RF of 0.44

Table legends

Table 1: Repeatability, Intra-Day and Inter-Day Precision data for Midodrine HCl
Table 2: Summary of validation parameters
Table: 3 Analysis of Tablet by HPTLC
Table: 4 Result of recovery study of Midodrine HCl
Table: 5 Analysis of Tablet by HPTLC

Table 1: Repeatability, Intra-Day and Inter-Day Precision data for Midodrine HCl

	Repeatability			Intraday Precision			Interday Precision		
Component	Mean area	S.D.	% RSD	Mean area	S.D.	% RSD	Mean area	S.D.	% RSD
Midodrine HCl	1250.92	1.53	0.12	1582.32	7.05	0.44	1584.09	8.12	0.51

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Table 2: Summary of validation parameters

Parameter		**Midodrine HCl**
Linearity range (ng/band)		200-1000
Correlation coefficient (R^2)		0.997
Precision	stability (n=6)(%RSD)	0.12
	Intraday precision(n=3) (%RSD)	0.43-0.39
	Interday precision(n=3) (%RSD)	0.49-0.64
Accuracy(%recovery)(n=3)		101.5-99.19
Limit of Detection		2.51 ng/band
Limit of Quantification		7.62 ng/band

Table: 3 Analysis of Tablet by HPTLC

Constituents	**Assay (%w/w)**	**Assay (mg)**	**%RSD**
Midodrine HCl	100.46	4.97	0.73

Table: 4 Result of recovery study of Midodrine HCl

Level	**Amountof Test taken (ng/band)**	**Amount of Std added (ng/band)**	**Amount Recovered ±SD (n=3)**	**%Recovery ±SD (n=3)**	**%RSD**
80%	400	320	321.25± 4.87	101.50	1.51
100%	400	400	412.27±7.27	102.26	1.76
120%	400	480	472.13±7.57	99.19	1.60

Table: 5 Analysis of Tablet by HPTLC

Sr. No.	**Type of degradation**	**PeakArea**	**No. of Degraded Peak**	**% Degradation**
Standard		1249.85	-	
1	Acid Degradation	1006.50	1	19.48%
2	Alkali Degradation	1040.37	1	16.72%
3	Oxidative Degradation	1098.01	-	12.75%
4	Thermal Degradation	1066.61	-	14.66%
5	Photolytic Degradation	1094.12	-	12.46%