Area under Curve Method Development and Validation of Midodrine Hydrocholride

Pritam S. Jain[1,*], Prashant V. Bhadane[2], Harshal P. Chaudhari[3], Sanjay J Surana[4]

R.C. Patel Institute of Pharmaceutical Education & Research, Karwand Naka, Shirpur, Dist. Dhule

***Corresponding Author:**
Email: pritash79@yahoo.com

ABSTRACT

A simple, rapid, accurate and economical Area under Curve method has been developed for estimation of Midodrine hydrochloride from bulk and pharmaceutical formulation. The λ max of Midodrine hydrochloride in water was found to be 289 nm. The drug follows linearity in the concentration range 12-84 μg/ml with correlation coefficient value 0.999. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 97.00% – 101.00% was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120%. The % recovery was found to be in the range 97.00% – 101.00%. The low values of % RSD are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 2 indicate that the method was precise. Ruggedness of the proposed method was studied with the help of two analysts. The above method was a rapid and cost-effective quality-control tool for routine analysis of Rizatriptan benzoate in bulk and in pharmaceutical dosage form.

Keywords: *Midodrine hydrochloride, UV-Spectrophotometry, Area under Curve, Validation.*

INTRODUCTION

Midodrine, (±)-1-(2′, 5′-dimethoxyphenyl)-2-glycinamido-ethanol. Midodrine Hydrochloride (Fig. 1) is a long acting α-adrenergic agonist that causes elevation of systemic blood pressure, accompanied by a reduction in heart rate.[1-3] Midodrine is a pro-drug of desglymidodrine (DMAE), developed by the attachment of the amino acid approach glycine to the functional amine of DMAE. It is therapeutically used, as a racemic (rac) mixture, for the treatment of orthostatic hypotension. The pro-drug Midodrine is primarily converted into its active metabolite desglymidodine after oral administration, mainly in the liver and in the systemic circulation by unknown peptidases.[3,4] After oral administration, Midodrine is rapidly absorbed. The plasma levels of the pro-drug peak after about half an hour, and decline with a half- life of approximately 25 minutes, while the metabolite reaches peak blood concentrations about 1 to 2 hours after a dose of Midodrine and has a half-life of about 3 to 4 hours.[5] Until now, the metabolism of Midodrine has not been extensively studied.[6]

Midodrine was developed by an amino-acid approach through the glycine promoiety attachment to the functional amine of desglymidodrine. Midodrine is a substrate for the intestinal H+ -coupled peptide transporter 1 (hPEPT1). This carrier mediated transport raises the bioavailability of Midodrine Hydrochloride to 93% when compared with 50% for desglymidodrine.[7]

Analysis part is an important from formulation development of any drug molecule. A suitable and validated method should be vacant for the drug delivery system for analysis of bulk drug, for release dissolution studies and estimation of drug in biological samples. The literature survey acknowledges that various methods for the determination of Midodrine hydrochlorides are noted. Some methods have been developed based on radioisotope-labeled techniques, high performance liquid chromatography (HPLC) with fluorescence and ultraviolet (UV) detection, and the capillary electrophoresis (CE). Hence, our study reports a simple, precise and economical UV- Spectrophotometric method for estimation of Midodrine Hydrochloride in bulk and tablet formulation. The method was validated according to ICH guidelines.[8]

EXPERIMENTAL WORK

Material and Method

Midodrine Hydrochloride working standard was obtained as gift sample from Ipca Pharmaceuticals, Mumbai. The drug was used without further purification. As the tablet formulation was not available in Indian market; tablet containing 5 mg Midodrine Hydrochloride were prepared in-house using direct compression technique. Prepared tablets were used as pharmaceutical formulation for further analysis.

Instrument

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; derivative mode: 1D (first order derivative, $dA/d\lambda$); band width ($\Delta\lambda$):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard stock and working standard solution

The standard stock solution of Midodrine Hydrochloride was prepared by dissolving accurately weighed 10mg of the drug in water and diluted to 100 ml with same solvent to obtain a final concentration of 84 µg/ml.

Method: Area under curve

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths $\lambda 1$ and $\lambda 2$. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The spectrum obtained of first order derivative was used to calculate AUC. The calibration curve was constructed by plotting concentration (12-84 µg/mL) versus AUC.

VALIDATION OF THE METHOD

The method was validated in terms of linearity, accuracy, precision, repeatability and ruggedness.

Linearity study

Different aliquots of Midodrine Hydrochloride in range 1.2-8.4 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with water to get concentrations 12, 24, 36, 48, 60, 72 and 84 µg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200-400 nm. The two wavelengths **278 and 299 nm** were selected for the determination of Area under Curve (AUC). The calibration plot was constructed as Area under Curve v/s concentration.

Accuracy (% Recovery)

To the pre analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120%. The solutions were reanalyzed by proposed method.

Precision

Precision of the method was studied as intraday and inter-day variations. Intra-day precision was determined by analyzing the 24, 36 and 48 µg/ml of Midodrine Hydrochloride solutions for three times in the same day. Inter-day precision was determined by analyzing the 24, 36 and 48 µg/ml of Midodrine Hydrochloride solutions daily for three days over the period of week.

LOD and LOQ (Sensitivity)

The sensitivity of measurements of Midodrine Hydrochloride by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and

LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n =3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability
Repeatability was determined by analyzing 48 µg/ml concentration of Midodrine Hydrochloride solution for six times.

Ruggedness
Ruggedness of the proposed method is determined for 48 µg/ml concentration of Midodrine Hydrochloride by analysis of dilution from homogenous slot by two analysts using same operational and environmental conditions.

DETERMINATION OF MIDODRINE HYDROCHLORIDE IN BULK
Accurately weighed 10 mg of Midodrine Hydrochloride was transferred to a 100 ml volumetric flask and 50 ml water was added. After shaking for 2min, the mixture was diluted up to mark with water. From stock solution correct dilution was taken in such a way that the final concentration is 84 µg/ml. The concentrations of the drug were calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 289 nm.

APPLICATION OF PROPOSED METHOD FOR PHARMACEUTICAL FORMULATION
For analysis of commercial formulation two tablets of 5 mg of Midodrine Hydrochloride was transferred to a 100 ml volumetric flask and 50 ml water was added. After ultrasonic vibration for 15 min, the mixture was diluted up to mark with water. The whole solution filtered using whatman filter paper no. 42. From filtrate correct dilution was taken in such a way that the final concentration is 84 µg/ml. The concentrations of the drug were calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 289 nm.

RESULTS AND DISCUSSION
Method Validation
The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Linearity studies:
The linear regression data for the calibration curves showed good linear relationship over the concentration range 12-84 µg/ml for Midodrine Hydrochloride (Fig. 1). Linear regression equation was found to be Y=0.0108+0.0051 (r^2 = 0.999). The result is expressed in Table 1.

Accuracy:
The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 2 which showed that the % amount found was between 97.00% to 101.00% with %R.S.D.>2.

Precision:
The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation (Table 3).

Sensitivity:
The linearity equation was found to be Y=0.0108+0.0051 (r^2 = 0.999). The LOQ and LOD for Midodrine Hydrochloride were found to be 1.5583 µg and 4.7222 µg, respectively (Table 4).

Repeatability:
Repeatability was determined by analyzing 48 µg/ml concentration of Midodrine Hydrochloride solution for six times and the % amount found was between 97% to 101% with % R.S.D. less than 2 (Table 5).

Ruggedness:
Peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 6**.** The result showed that the % R.S.D. was less than 2%.

Determination of Midodrine Hydrochloride in bulk:
The concentrations of the drug were calculated from linear regression equations. The % amount found was between 97.00% to 101.00% (Table 7).

Application of proposed method for pharmaceutical formulation:
The spectrum was recorded at 289 nm. The concentrations of the drug were calculated from linear regression equation. The % amount was found between 97.00% to 101.00% (Table 8).

Table 1: Linearity study of Midodrine hydrochloride

Concentration µg/mL	Absorbance,[a] mean± SD (n=6)	% RSD
12	0.1373 ± 0.0015	1.1236
24	0.2648 ± 0.0024	0.9353
36	0.3893 ± 0.0038	0.9875
48	0.5245 ± 0.0015	0.2915
60	0.6553 ± 0.0037	0.5666
72	0.7903 ± 0.0044	0.5671
84	0.9089 ± 0.0089	0.9805

(n= no. of estimations)

Table 2: Recovery studies

Drug	Initial amount (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL, n=3)	% Recovered	% RSD
Midodrine hydrochloride	36	28.8	28.1265	97.6616	1.6569
	36	36	36.2098	100.5830	1.5214
	36	43.2	43.2098	100.0229	0.2511

(n= no. of estimations)

Table 3: Results of Precision studies

Conc. µg/mL	Intra-day[a]		Inter-day[a]	
	Amt. found	% RSD	Amt. found	% RSD
24	24.20	0.5964	24.15	1.1120
36	35.25	0.9293	35.39	0.9745
48	48.23	0.5292	47.99	0.3184

[a]Average of three estimation

Table 4: Sensitivity studies

LOD(µg/mL)	LOQ(µg/mL)
1.5583	4.7222

Table 5: Repeatability Studies

Component	Amount taken (µg/mL) (n=6)	Amount found[a] (%)	% RSD
Midodrine Hydrochloride	48	48.12 ± 0.17	0.36

[a]Average of six estimations.

Table 6: Ruggedness study

Component	Amount taken (µg/mL) (n=3)	Amount found (%)[a]	
		Analyst I ± SD	Analyst II ± SD
Midodrine Hydrochloride	48	99.4213 ± 0.2249	99.9357 ± 0.2952

Table 7: Analysis of Midodrine hydrochloride in Bulk

Concentration (µg/mL)	Amount found (µg)	Amount found (%)
84	82.7037	98.4567
	84.5925	100.7055
	83.3240	99.1953
	84.6111	100.7275
	83.6944	99.6362
	83.3888	99.2724
Mean ± SD	83.7191 ± 0.7556	99.6656 ± 0.8996
% RSD	0.9026	0.9026

Table 8: Analysis of Midodrine hydrochloride in Formulation

Concentration (µg/mL)	Amount found (µg)	Amount found (%)
84	82.5925	98.3245
	82.3518	98.0379
	83.9166	99.9001
	83.9722	99.9669
	83.1388	98.9748
	83.1944	99.0410
Mean ± SD	83.1944 ± 0.6635	99.0410 ± 0.7899
% RSD	0.7976	0.7976

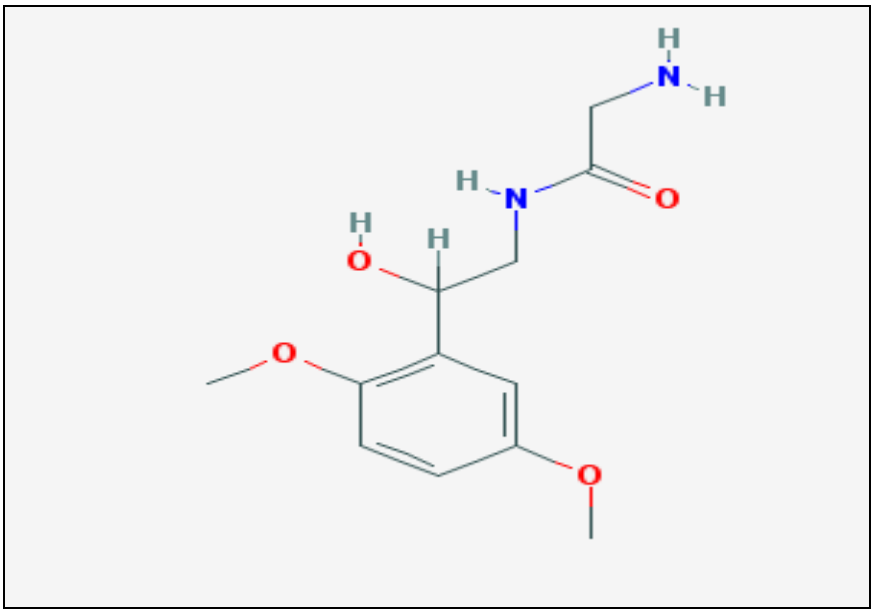

Fig. 1 Chemical structure of Midodrine hydrochloride

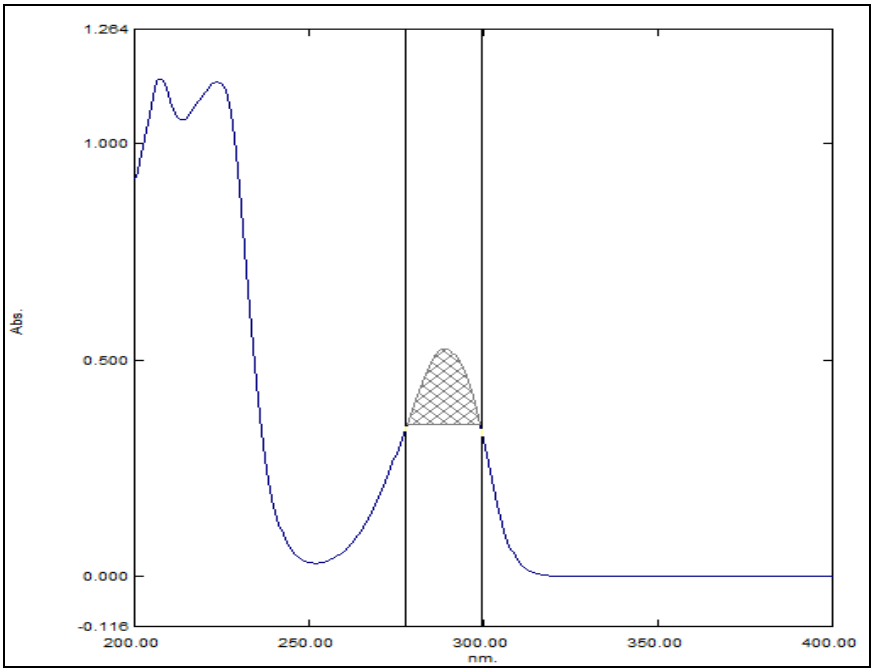

Fig. 2: Area under curve spectrum of Midodrine Hydrochloride in water

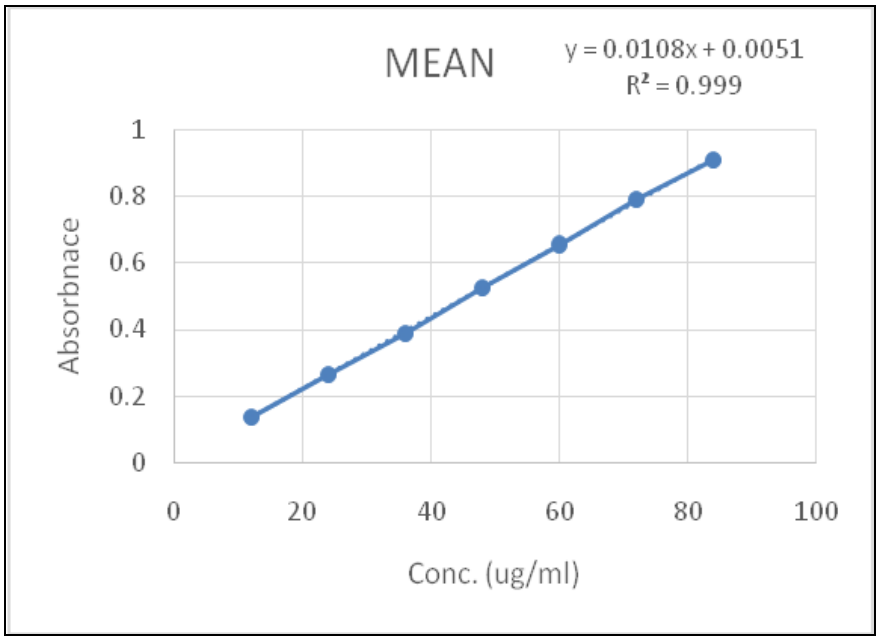

Fig. 3: Calibration curve of Midodrine Hydrochloride

CONCLUSION

This UV Spectrophotometric method is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Midodrine Hydrochloride in tablet formulation. The validation procedure confirms that this is an appropriate technique for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

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