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Development and Validation of Zero and First-Order Derivative Area under Curve Spectrophotometric Methods for the Determination of Midodrine Hydrochloride in Bulk Material and in Pharmaceutical Dosage Form

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Abstract

Aim: The aim of this work is to establish two simple, economical, and rapid spectrophotometric methods for the quantification of Midodrine Hydrochloride in bulk material and in tablets. Further, this study is designed to validate the developed methods as per ICH guidelines.

Materials and Methods: In Methods I and II, a stock standard solution was prepared by dissolving 10 mg of Midodrine Hydrochloridein 100 mL of 10% *v/v* water to obtain a concentration of 100 µg/mL. After suitable dilution, 10 µg/mL of Midodrine Hydrochloride was prepared and scanned in the UV-visible range 400-200 nm; Midodrine Hydrochloride showed a maximum wavelength at 289 nm. In Method I, Area under Curve (AUC) of the zero-order spectrum was recorded between 278.00 and 299.00 nm. While, in Method II, zero-order spectra were derivative into first-order and the AUC was recorded between 228.00 and 237.50 nm. For a linearity study, series of dilutions were prepared from stock solutions.

Results: In Method I, and II, Midodrine Hydrochloride followed linearity in the concentration range of 12-84 µg/mL with (r^2>0.999).

Conclusion: The developed methods are simple, precise, rugged, robust, and economical. Both these methods can be used for routine analysis of Midodrine Hydrochloride from its tablet formulation.

Keywords: Area under curve spectrophotometry; Derivative-spectrophotometry; Midodrine Hydrochloride

Introduction

Midodrine, (±)-1-(2′,5′-dimethoxyphenyl)-2-glycinamido-ethanol.Midodrine Hydrochloride (Figure 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 desglymidodrine 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].

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].

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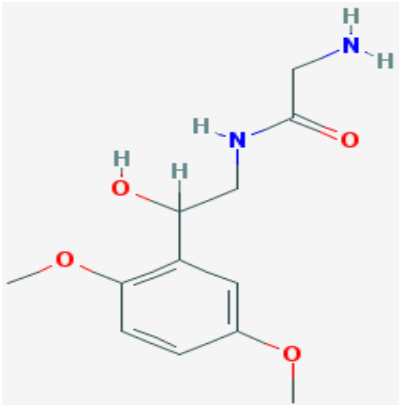

Figure 1: Chemical structure of Midodrine Hydrochloride.

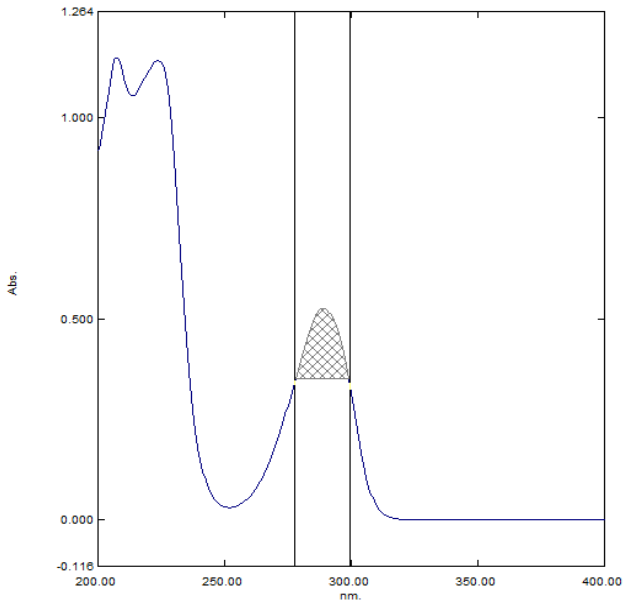

Figure 2: UV-spectrum of Midodrine Hydrochloride in water.

Materials and Methods

Materials

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 a computer loaded with spectra manager software UV Probe 2.21 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. All weights were taken on an electronic balance (Model Shimadzu AUX 120).

Preparation of stock standard solution andselection of wavelengths

A stock standard solution was prepared by dissolving 10 mg of Midodrine Hydrochloride in a 100 mL of 10% *v/v* water to obtain a concentration of 100 µg/mL. From it, an appropriate concentration of 10 µg/mL was prepared and scanned in the UV-visible range 400-200 nm; Midodrine Hydrochloride showed a maximum absorbance at 289 nm. In Method I, Area under Curve (AUC) of the zero-order spectrum was recorded between the 278.00 and 299.00 nm. While, in Method II, zero-order spectra were derivative into first-order and the AUC was recorded between 228.00 and 237.50 nm.

Validation of the method

Study of linearity curves: From the stock standard solution, an appropriate amount of aliquots portion in the range of 1.2-8.4 mL were transferred into a series of 10 mL volumetric flasks and diluted up to mark using the same solvent to obtain a concentration in the range of 12-84 µg/mL. The solutions were scanned on a spectrophotometer in the range of 400-200 nm. The calibration curves were plotted concentrations *versus* AUC between 278.00 nm and 299.00 nm (Method I). While in Method II, an appropriate amount of aliquots portion in the range of 1.2-8.4 mL were transferred into a series of 10 mL volumetric flasks and diluted up to the mark using the same solvent to obtain a concentration in the range of 12-84 µg/mL. The calibration curve was plotted as concentration *versus* AUC between 228.00 and 237.50 nm (Method II).

Recovery studies: To the pre-analyzed sample solutions, a known amount of stock standard solution was added at different levels, i.e. 80%, 100%, and 120%. The solutions were re-analyzed by the proposed methods.

Precision: The precision of the methods was studied as intra-day and inter-day variations. In Method I, precision was determined by analyzing the 24, 36, and 48 µg/mL of Midodrine Hydrochloride solutions as intra-day and inter-day variations. In Method II, precision was determined by analyzing the 24, 36, and 48 µg/mL of Midodrine Hydrochloride solutions as intra-day and inter-day variations.

Sensitivity: The sensitivity of measurements of Midodrine Hydrochloride by the use of the proposed methods was estimated in terms of the Limit of Quantification (LOQ) and the Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD=3.3 × *N*/*B* and LOQ=10 × *N*/*B*, where '*N*' is the standard deviation of the AUC 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 and 48 µg/mL concentrations of Midodrine Hydrochloride solution for six times for Methods I and II, respectively.

Ruggedness: The ruggedness of the proposed methods was determined for 48 µg/mL concentrations of Midodrine Hydrochloride by analysis of aliquots from a homogenous slot by two analysts using the same operational and environmental conditions for Methods I and II, respectively.

Application of proposed method for pharmaceutical formulation: Twenty tablets were accurately weighed, average weight determined and ground into fine powdered. A quantity of powder

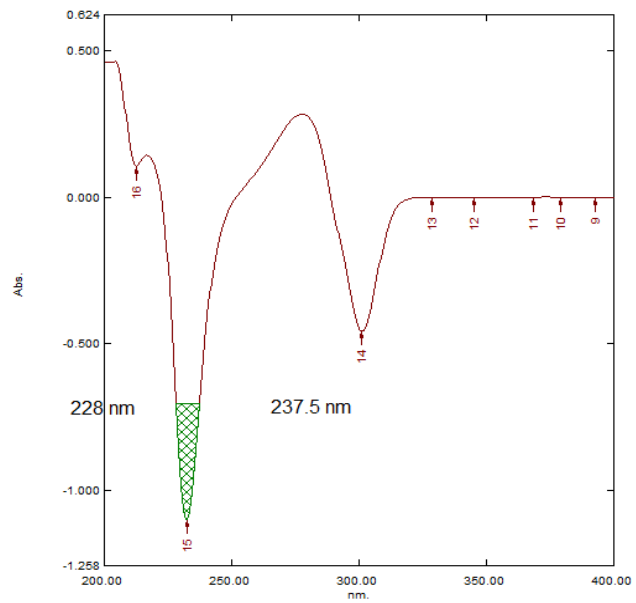

Figure 3: First-order derivative spectrum of Midodrine Hydrochloride in water.

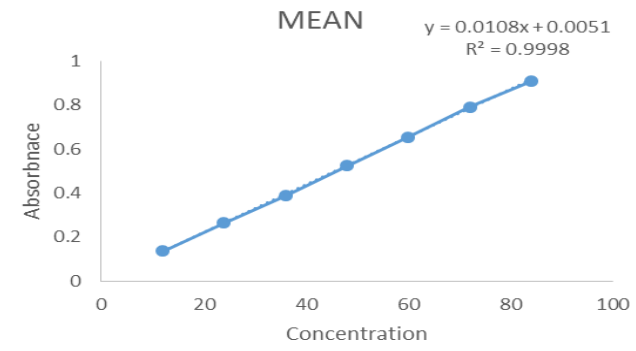

Figure 4: Linearity curve of Midodrine Hydrochloride by AUC.

equivalent to one tablet was transferred into a 100 mL volumetric flask containing 30 mL of water; the volume was adjusted to the mark using the same solvent. An appropriate volume 4.8 mL was transferred into a 10 mL volumetric flask and the volume were adjusted to the mark to obtain the desired concentration of 48 µg/mL. The AUC was recorded at selected wavelengths for Method I. While in Method II, AUC of the first-order derivative spectrum was recorded in between selected wavelength ranges. The concentration of the drug was determined from the respective linear regression equations.

Table 1: Optical characteristics and linearity data of Midodrine Hydrochloride.

Parameters	Method I	Method II
Linearity range (µg/mL)	12-84	12-84
Selected range (nm) for AUC	278-299	228.0-237.5
Slope	0.0108	0.043
Intercept	0.0051	0.064
Correlation coefficient	0.999	0.999
Limit of detection (µg)	0.0567	0.1760
Limit of quantitation (µg)	0.1721	0.5333

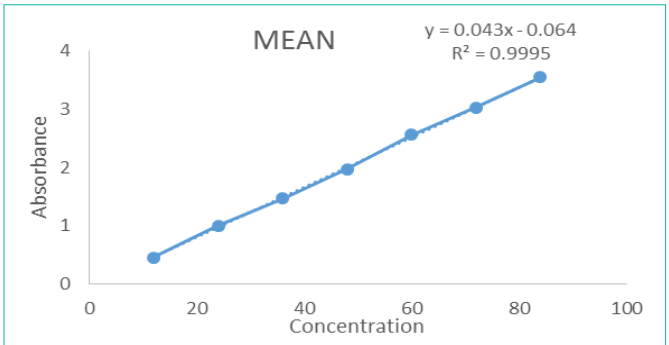

Figure 5: Linearity curve of Midodrine Hydrochloride by first order derivatives.

Determination of Midodrine Hydrochloride in bulk: A quantity of powder equivalent to one tablet was transferred into a 100 mL volumetric flask containing 30 mL of water; the volume was adjusted to the mark using the same solvent. An appropriate volume 4.8 mL was transferred into a 10 mL volumetric flask and the volume were adjusted to the mark to obtain the desired concentration of 48 µg/mL. The AUC was recorded at selected wavelengths for Method I. While in Method II, AUC of the first-order derivative spectrum was recorded in between selected wavelength ranges. The concentration of the drug was determined from the respective linear regression equations.

Results and Discussion

Selection of wavelengths

Figures 2 and 3 show the selection of wavelengths in Methods I and II, respectively. The selection of wavelengths in both the methods is based on there producibility of the results.

Linearity studies

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 12-84 µg/mL for Method I and 12-84 µg/mL for Method II (Figures 4 and 5). The results are expressed in (Table 1).

Accuracy

The pre-analyzed sample used in Methods I and II was 4 and 10 µg/mL, respectively. In Method I, the mean % recovery was found to be in the range of 97.66-100.02%. While in Method II, it was found to be in the range of 99.61-99.69%. The results are expressed in (Table 2).

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 indicate that the methods were precise for the determination of drugs in formulation. The results are expressed in (Table 3).

Sensitivity

The LOD and LOQ for Midodrine Hydrochloride were found to be 0.0567 and 0.1721 µg, respectively, for Method I. For Method II, they were found to be 0.1760 and 0.5333 µg, respectively. The results are expressed in (Table 1).

Repeatability

Repeatability was determined by analyzing 48 µg/mL (Method

Table 2: Accuracy.

% Value	Initial amount (µg/mL)	Amount added (µg/mL)	Method I (n=3)		Method II (n=3)	
			% Recovery	% RSD	% Recovery	% RSD
80	36	28.8	97.6616	1.6569	99.6177	0.1238
100	36	36	100.5830	1.5214	99.4186	0.1299
120	36	43.2	100.0229	0.2511	99.6985	0.0539

Table 3: Precision.

Conc. (µg/mL)		Intra-day[a]		Inter-day[a]	
Method I	Method II	% RSD		% RSD	
		Method I	Method II	Method I	Method II
24	24	0.5964	0.1838	1.1120	0.2002
36	36	0.9293	0.2867	0.9745	0.2415
48	48	0.5292	0.1088	0.3184	0.1748

[a]Average of three estimates

Table 4: Ruggedness.

Method	Amount taken (µg/mL) (n=3)	Amount found (%)[a]	
		Analyst I	Analyst II
Method 1	48	99.4213	99.9357
Method 2	48	99.0310	99.0633

[a]Average of Six estimations

Table 5: Repeatability.

Method	Amount taken (µg/mL) (n=6)	Amount found[a] (%)	% RSD
Method 1	48	100.4405	0.3693
Method 2	48	99.0552	0.0861

[a] Average of six estimations.

Table 6: Analysis of bulk.

Method	Concentration (µg/mL) (n=6)	Amount found (µg/mL)(n=6)	Amount found[a] (%)	% RSD
Method 1	48	47.7592	99.4984	0.1025
Method 2	48	47.5542	99.0713	0.1214

[a]Average of six estimations.

Table 7: Analysis of formulation brand name-Lefra 10mg (Torrent).

Method	Concentration (µg/mL) (n=6)	Amount found (µg/mL)(n=6)	Amount found[a] (%)	% RSD
Method 1	48	47.8703	99.7299	0.1173
Method 2	48	47.0620	98.0458	0.1196

[a]Average of six estimations.

I) and (Method II) concentrations of Midodrine Hydrochloride solution for six times and the % amount determined with % RSD<2 for both the methods. The results are expressed in (Table 5).

Ruggedness

The peak area was measured for the same concentration solutions, six times for both methods. The results were in the acceptable range for both the drugs. The results showed that the % RSD was less than 2% (Table 4).

Determination of Midodrine Hydrochloride in bulk: The concentrations of the drug were calculated from linear regression equations. The % amount found was between 97.50% to 100.31% both Method 1 and Method 2 (Table 6).

Application of proposed method for pharmaceutical formulation: The spectrum was recorded at 261 nm. The concentrations of the drug were calculated from linear regression equation. The % amount was found between 97.50% to 100.31% both Method 1 and Method 2 (Table 7).

Conclusion

Both the developed methods are economical, simple, accurate, precise and rugged, and can be used for the usual study of Midodrine Hydrochloride from its pharmaceutical formulations. The methods are developed for quantification of Midodrine Hydrochloridein tablets. It is also used in routine quality control of the formulations containing Midodrine Hydrochloride.

Acknowledgment

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