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QUANTITATIVE ESTIMATION OF MIDODRINE HYDROCHLORIDE BY RP-HPLC AND UV-SPECTROPHOTOMETRY METHODS

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Abstract:
Four methods RP–HPLC and UV–Spectrophotometry (Zero Order – AUC, First Order Derivative using Amplitude and First Order Derivative using AUC) have been developed for determination of Midodrine Hydrochloride in bulk and tablets. RP – HPLC method are found to be accurate, precise, rugged and sensitive. A simple, sensitive, precise and accurate RP – HPLC method for the determination of Midodrine Hydrochloride both bulk and pharmaceutical formulation has been developed and validated as per the International Conference on Harmonization (ICH) guidelines. The % RSD value for intra – day and inter – day precision was found to be in the range of 0.45 – 1.37 % and 1.48 – 1.55 %. The mean % recovery was found to be in the range of 98.87 – 99.59 %. The low values of LOD (0.09 µg) and LOQ (0.28 µg) indicate high sensitivity of the method. The developed method can routinely be used for analysis of Midodrine Hydrochloride in pharmaceutical formulations. Three simple, rapid, accurate, precise, reliable and economical UV – Spectrophotometry methods have been proposed for the determination of Midodrine Hydrochloride in bulk and in pharmaceutical formulation. Method 2 is zero order UV – Spectrophotometry using Area Under Curve Method, Method 3 is first order derivative UV – Spectrophotometry using Amplitude and Method 4 first order derivative UV – Spectrophotometry using Area Under Curve Method. The developed methods have shown good results in terms of linearity, accuracy, precision and sensitivity for bulk drug and marketed formulation as well.

Key words: *Midodrine hydrochloride, HPLC, UV-Spectrophotometry, Method Development, validation*

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INTRODUCTION:

Pharmaceutical Analysis is the branch of pharmacy that is responsible for developing sensitive, reliable and more accurate method for the estimation of drugs in pharmaceutical dosage forms and in biological system. Pharmaceutical Analytical techniques are applied mainly in two areas, viz Quantitative Analysis and Qualitative Analysis. Analytical Chemistry is defined as the "Science and the Art of determining the composition of materials in terms of the elements or compounds contained"[1]. This branch of chemistry, which is both theoretical and a practical science, is practiced in a large number of laboratories in many diverse ways while analytical method, is a specific application of a technique to solve an analytical problem. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species, that is, to find out what a substance is composed and exactly how much[2].

Since first initiated by the U.S. Food and Drug Administration (FDA) in its "Pharmaceutical cGMPs for the twenty-first century" (Quality by design) has become an important concept for the pharmaceutical industry that is further defined in the International Conference on Harmonization (ICH) guidance on pharmaceutical development as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management"[3]. Recently, increased attention has been paid to Quality by design within the pharmaceutical industry to actively seek out quality using its underlying principles. As analytical techniques and methods are used for the quality control of pharmaceutical compounds and thereby assure patient safety and efficacy, they have become an essential part of pharmaceutical Quality by design. The topics around what is Quality by design for analytical methods or how to apply Quality by design to analytical method development have been discussed in the recent literature[4]. Building-in quality as the method is developed can be achieved by defining the method objectives at the beginning of the method development effort, by using sound development tools and by applying analytical sciences knowledge to anticipate and pre-empt problems. The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified. This approach ensures a very high likelihood of method success during the product lifecycle. Thus, the validation which is usually performed after method development will serve the purpose of confirming method performance as opposed to identifying potential problem areas[5].

Midodrine Hydrochloride is indicated for the treatment of symptomatic orthostatic hypotension (OH). Because Midodrine Hydrochloride can cause marked elevation of supine blood pressure (BP > 200 mmHg systolic), it should be used in patients whose lives are considerably impaired despite standard clinical care, including nonpharmacologic treatment (such as support stocking), fluid expansion, and lifestyle alterations. The indication is based on Midodrine Hydrochloride's effect on increase in 1 - minute standing systolic blood pressure, a surrogate marker considered likely to correspond to a clinical benefit. At present, however, clinical benefits of Midodrine Hydrochloride principally improved ability to perform life activities, have not been established[6]. A detailed literature survey for Midodrine Hydrochloride revealed that few chromatographic and spectroscopic methods for estimation of Midodrine Hydrochloride in bulk and pharmaceutical formulation. As far there is no any RP–HPLC and Area Under Curve UV method is reported. The method was validated according to ICH guideline [7-8].

MATERIAL AND METHODS:

Reagents and Chemicals

Midodrine Hydrochloride working standard was obtained as gift sample from Ipca Pharmaceuticals, Mumbai. The drug was used without further purification. Analytical grade solvents and reagents were purchased from Merk specialties Pvt.Ltd. Mumbai (M.S). Double distilled water with filtration through membrane filter was used. 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.

Instrumentation

RP–HPLC chromatographic system is of Shimadzu (Japan) liquid chromatography comprising LC-20 AD solvent delivery system (pump), SPD-M20A Photo Diode Array Detector, Data processor LC Solution and a Rheodyne injector with 20 µL loop. Prior to chromatography the mobile phase was filtered using 0.45 µm membrane filter and degassed by ultrasonic vibrations. All experiments were carried out at 35ºC and the flow rate of the mobile phase was kept at 1.0 mL/min.

Methods I (HPLC Method)

Optimization of mobile phase strength

For optimization of mobile phase; initially methanol and water in various proportions were tried but the splitting of the peak was observed. Therefore, pH of aqueous phase was adjusted using *ortho-phosphoric acid*. Finally, mobile phase consisting of Methanol : Water (60:40%*v/v*) pH of aqueous mobile phase adjusted to 6.0 ± 0.02 with *ortho-phosphoric acid* and a column temperature of 35ºC show good resolution and symmetric peak for Midodrine Hydrochloride. The flow rate of mobile phase was adjusted to 1.0 ml/min. under these optimum chromatographic conditions, the retention time for Midodrine Hydrochloride was found to be 4.54 ± 0.02 min when detection was carried out at 289 nm.

Linearity Studies

From the stock standard solution, aliquots of Midodrine Hydrochloridein range 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with water to get concentrations 1, 2, 3, 4, 5, 6 and 7 µg/ml, respectively. A constant volume 20 µL of Midodrine Hydrochloride was injected into column with the help of microliter syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *versus* thedrug concentration.

Analysis of Bulk Material

Accurately weighed 10 mg of Midodrine Hydrochloridewas transferred to a 10 ml volumetric flask containing 8 ml mobile phase and volume was adjusted up to mark. It was further diluted with mobile phase to get concentration 3µg/mL of Midodrine Hydrochloride. Constant volume 20 µL was injected into column and peak area is recorded. The concentration of Midodrine Hydrochloride was determined from linear regression curve. The procedure was repeated for six times.

Analysis of tablet formulation

For analysis of inhouse formulation, ten tablets of 5 mg of Midodrine Hydrochloride was transferred to a 100 ml volumetric flask containing 40 ml mobile phase, shaken manually for 15 min; volume was adjusted to mark using same solvent. The solution was then filtered through Whatmann filter paper no. 41giving concentrations of stock solutions 100 µg/mL. From the filtrate, an appropriate volume of solution was diluted to get final concentration of 3 µg/mL using mobile phase. A constant volume of 20 µL was adjusted into column and concentration of Midodrine Hydrochloride was determined from linear regression curve. **Validation**

The method was validated as per the ICH guidelines in terms of recovery, precision, ruggedness and sensitivity.

Recovery Studies

Accuracy is determined by applying the method to the pre-analyzed samples in which 80 %, 100 %, 120 % of the standard have been added. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. The accuracy studies were repeated for 3 times at each level; % RSD was calculated.

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). Precision was studied as repeatability, intra-day and inter-day precision. Repeatability of sample measurement of area was carried out using six replicates of the same concentrations (3µg/mL of Midodrine Hydrochloride). The intra-day and inter-day variation for the determination of Midodrine Hydrochloride was carried out at three different concentrations levels of 2, 3 and 4µg/mL.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**.**

Ruggedness

Ruggedness of the method was performed by two different analytes keeping experimental and environmental conditions same. The concentration used to performed ruggedness was 3µg/mL.

Sensitivity

The quantitation limit is a parameter of quantitative assay for low levels of compounds in simple matrices, and is used particularly for the determination impurities and/or degradation products. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formulae. LOD = 3.3 * (ASD)/S; LOQ = 10* (ASD)/S; where, ASD = Average Standard deviation of response, S = the slope of the calibration. To study the LOD and LOQ of Midodrine Hydrochloride, lower part of the linearity curve was considered. Different concentration i.e. 1, 2, 3, 4, 5, 6 and 7µg/mL were selected for the method. The Average Standard deviation of peak areas was determined. For determination of LOD and LOQ slope of corresponding calibration curve was considered. LOD and LOQ were found to be 0.09 µg and 0.28µg for Midodrine Hydrochloride.

Methods II (UV Method)

Determination of wavelength

From the stock standard solution, an appropriate volume 1.0 ml was transferred into 10 ml volumetric flask, diluted to mark with water to give concentration of 10µg/ml. The resulting solution was scanned in UV range (400 nm – 200 nm). Zero order spectrum, obtained using UV probe 2.21 software of the instrument. The two wavelengths 278.00 – 299.00 nm were selected for the determination of Area Under Curve (AUC).

Linearity study

An appropriate volume in the range of 1.2-8.4 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with methanol to get concentrations 12, 24, 36, 48, 60, 72 and 84 µg/ml. It was scanned in the UV range and derivatized into first order spectrum. The solutions were scanned on spectrophotometer in the UV range 400 – 200 nm and zero order spectrum obtained. AUC measured was between the chosen wavelengths and a calibration curve was constructed AUC *versus* concentration.

Analysis of Midodrine Hydrochloride in Bulk

Accurately weighed 10 mg of Midodrine Hydrochloride was transferred into 100 ml volumetric flask containing 50 ml water and volume was made up to the mark using same. Appropriate volume 1.0 ml of this solution was transferred in to 10 ml volumetric flask and volume was adjusted to mark using same solvent to get final concentration of about 10 µg/ml of Midodrine Hydrochloride and AUC was measured at selected wavelengths.

Analysis of Midodrine Hydrochloride Tablets

For analysis of inhouse formulation, 10 tablets of Midodrine Hydrochloride were weighed and ground into fine power. A quantity of powdered drug equivalent to 10 mg was transferred into 100 ml volumetric flask containing 50 ml of water and the volume was made up to the mark with same solvent. An appropriate volume 4.8 ml was diluted to 10 ml (i.e., 48 µg/ml). It was scanned in the UV-range, the analysis was repeated for six times and concentration was determined from linearity equation.

Validation of the Method

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

Accuracy

To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80 %, 100 % and 120 %. Amplitude was measured for each concentration at selected wavelength. High recovery and low standard deviation confirmed that proposed method is accurate for determination of Midodrine Hydrochloride.

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing 24, 36, and 48 µg/ml of Midodrine Hydrochloride for three times in the same day.

Inter-day precision was determined by analyzing the Midodrine Hydrochloride daily for three consecutive days over a period of week using same concentrations.

Repeatability Study

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

Sensitivity

Sensitivity of the proposed method was estimated in terms of LOD and LOQ. The LOD and LOQ were calculated by the use of equation, LOD = 3.3 (SD/S) and LOQ = 10 (SD/S); Where, SD is the residual standard deviation of the peak areas of the drug (n = 6) and 'S' is the slope of the line.

Sensitivity was performed between 12 - 24 µg/ml. The first order derivative was recorded at selected wavelength**.**

Ruggedness

Ruggedness of the proposed method was determined by analyzing 48 µg/ml concentration of Midodrine Hydrochloride by two different analysts using similar operational and environmental conditions**.**

Methods III (UV Method, First order)

Preparation of Stock Standard Solutions of Midodrine Hydrochloride

The stock standard solution was prepared by dissolving 10 mg of Midodrine Hydrochloride in 100 ml of water to obtain concentration 100 µg/ml.

Determination of wavelength

From the stock standard solution, an appropriate volume 1.0 ml was transferred into 10 ml volumetric flask, diluted to mark with water to give concentration of 10 µg/ml. The resulting solution was scanned in UV range (400 nm – 200 nm). In spectrum Midodrine Hydrochloride showed absorbance maximum at 289 nm. This zero order absorption spectra derivatized in first order by using UV-Probe 2.21 software with delta lambda 05 and scaling factor 10. The amplitude of the trough was found to be 232 nm.

Linearity study

An appropriate volume in the range of 1.2-8.4 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with methanol to get concentrations 12, 24, 36, 48, 60, 72 and 84 µg/ml. It was scanned in the UV range and derivatized into first order spectrum. Amplitude for each concentration at selected wavelength was recorded and calibration curve was plotted, Amplitude *versus* Concentration.

Analysis of Midodrine Hydrochloride in Bulk

Accurately weighed 10 mg of Midodrine Hydrochloride was transferred into 100 ml volumetric flask containing 50 ml water and volume was made up to the mark using same. Appropriate volume 1.0 ml of this solution was transferred in to 10 ml volumetric flask and volume was adjusted to mark using same solvent to get final concentration of about 10 µg/ml of Midodrine Hydrochloride. Earlier adopted procedure was followed and amplitude was measured at selected wavelength.

Analysis of Midodrine Hydrochloride Tablets

For analysis of inhouse formulation 10 tablets of Midodrine Hydrochloride were weighed and ground into fine power. A quantity of powdered drug equivalent to 10 mg was transferred into 100 ml volumetric flask containing 50 ml of water and the volume was made up to the mark with same solvent. An appropriate volume 4.8 ml was diluted to 10 ml (i.e., 48 µg/ml). It was scanned in the UV-range, the spectrum was derivatized into first order and amplitude was recorded for each concentration. The analysis was repeated for six times and concentration was determined from linearity equation.

Validation of the Method

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

Accuracy

To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80 %, 100 % and 120 %. Amplitude was measured for each concentration at selected wavelength. High recovery and low standard deviation confirmed that proposed method is accurate for determination of Midodrine Hydrochloride.

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing 24, 36, and 48 µg/ml of Midodrine Hydrochloride for three times in the same day.

Inter-day precision was determined by analyzing the Midodrine Hydrochloride daily for three consecutive days over a period of week using same concentrations. The results are shown in **Table 27.**

Repeatability Study

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

Sensitivity

Sensitivity of the proposed method was estimated in terms of LOD and LOQ. The LOD and LOQ were calculated by the use of equation, LOD = 3.3 (SD/S) and LOQ = 10 (SD/S); Where, SD is the residual standard deviation of the peak areas of the drug (n = 6) and 'S' is the slope of the line.

Sensitivity was performed between 12 - 24 µg/ml. The first order derivative was recorded at selected wavelength**.**

Ruggedness

Ruggedness of the proposed method was determined by analyzing 48 µg/ml concentration of Midodrine Hydrochloride by two different analysts using similar operational and environmental conditions**.**

Methods IV (UV Method, First order, Area under curve)

Selection of Solvent

Solubility of Midodrine Hydrochloride was checked in different solvents and water was selected as solvent.

Preparation of Stock Standard Solutions of Midodrine Hydrochloride

The stock standard solution was prepared by dissolving 10 mg of Midodrine Hydrochloride in 100 ml of water to obtain concentration 100 µg/ml.

Determination of wavelength

An appropriate volume 1.0 ml of standard stock solution of Midodrine Hydrochloride was transferred into 10 ml volumetric flask, diluted to mark with water to give concentration of 10 µg/ml. The resulting solution was scanned in UV range (400 nm – 200 nm). Zero order spectrum is derivatized into first order spectrum, using UV probe 2.21 software of the instrument using delta lambda 05 and scaling factor 10. Two wavelengths 228.00 and 237.50 nm were selected for the determination of AUC.

Linearity study

An appropriate volume in the range of 1.2-8.4 ml were transferred into series of 10 ml volumetric

flasks and the volume was made up to the mark with methanol to get concentrations 12, 24, 36, 48, 60, 72 and 84 µg/ml. It was scanned in the UV range and derivatized into first order spectrum. Amplitude for each concentration at selected wavelength was recorded and calibration curve was plotted, Amplitude *versus* Concentration.

Analysis of Midodrine Hydrochloride in Bulk
Accurately weighed 10 mg of Midodrine Hydrochloride was transferred into 100 ml volumetric flask containing 50 ml water and volume was made up to the mark using same. Appropriate volume 1.0 ml of this solution was transferred in to 10 ml volumetric flask and volume was adjusted to mark using same solvent to get final concentration of about 10 µg/ml of Midodrine Hydrochloride. Earlier adopted procedure was followed and amplitude was measured at selected wavelength**.**

Validation of the Method
The method was validated in terms of linearity, accuracy, precision and ruggedness.

Accuracy
To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80 %, 100 % and 120 %. Amplitude was measured for each concentration at selected wavelength. High recovery and low standard deviation confirmed that proposed method is accurate for determination of Midodrine Hydrochloride. **.**

Precision
Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing 24, 36, and 48 µg/ml of Midodrine Hydrochloride for three times in the same day.

Inter-day precision was determined by analyzing the Midodrine Hydrochloride daily for three consecutive days over a period of week using same concentrations.

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

Sensitivity
Sensitivity of the proposed method was estimated in terms of LOD and LOQ. The LOD and LOQ were calculated by the use of equation, LOD = 3.3 (SD/S) and LOQ = 10 (SD/S); Where, SD is the residual standard deviation of the peak areas of the drug (n = 6) and 'S' is the slope of the line.
Sensitivity was performed between 12 - 24 µg/ml. The first order derivative was recorded at selected wavelength.

Ruggedness
Ruggedness of the proposed method was determined by analyzing 48 µg/ml concentration of Midodrine Hydrochloride by two different analysts using similar operational and environmental conditions and results are shown in Table.

RESULTS AND DISCUSSION:

Development and Validation of RP – HPLC Method for determination of Midodrine Hydrochloride in Bulk & Tablets

A simple, sensitive, precise and accurate RP – HPLC method for the determination of Midodrine Hydrochloride both bulk and pharmaceutical formulation has been developed and validated as per the International Conference on Harmonization (ICH) guidelines. The chromatographic separation was achieved on Princeton SPHER100 C18 column (4.6 x 250mm, 5µ), detection at 289 nm using Methanol: Water (pH adjusted to 6.0 with Ortho-phosphoric acid) 60:40 % *v/v* as *mobile phase*. A typical retention time for Midodrine Hydrochloride was 4.54 ± 0.02 min. Linearity was observed in range of concentration range of 1 – 6µg/mL with coefficient correlation (r2 = 0.998). The % RSD value for intra – day and inter – day precision was found to be in the range of 0.45 – 1.37 % and 1.48 – 1.55 %. The mean % recovery was found to be in the range of 98.87 – 99.59 %. The low values of LOD (0.09 µg) and LOQ (0.28 µg) indicate high sensitivity of the method. The developed method can routinely be used for analysis of Midodrine Hydrochloride in pharmaceutical formulations.

Development and Validation of UV – Spectrophotometry – Zero order using AUC, First order derivative using Amplitude, First order derivative using AUC Method for determination of Midodrine Hydrochloride in Bulk and Tablets

Three simple, rapid, accurate, precise, reliable and economical UV – Spectrophotometry methods have been proposed for the determination of Midodrine Hydrochloride in bulk and in pharmaceutical formulation. Method 2 is zero order UV – Spectrophotometry using Area Under Curve Method, Method 3 is first order derivative UV – Spectrophotometry using Amplitude and Method 4 first order derivative UV – Spectrophotometry using Area Under Curve Method. The developed methods

have shown good results in terms of linearity, accuracy, precision and sensitivity for bulk drug and marketed formulation as well. In water, Midodrine Hydrochloride showed maximum absorbance at 289 nm. For Method 2 absorbance was recorded at 289 nm while for Method 2 area under curve was integrated in the wavelength range of 278.00 – 299.00. For Method 3 amplitude was measured at 232 nm while for Method 4 area under curve was selected in the wavelengths range of 228.00 – 237.50 nm. For Method 2, 3, and 4 Midodrine Hydrochloride obeyed Lambert – Beer's law in the range of 12 – 84 µg/ml and the correlation coefficient were found to be > than 0.999.

Table 1: Summary of Method 1

Parameter	RP – HPLC METHOD
Linearity Range	1 – 6
Correlation coefficient	0.9994
LOD	0.09
LOQ	0.28
Accuracy (n= 3), % RSD	0.38
Precision (% RSD)	
Repeatability (n = 6)	0.41
Intra – day (n = 3)	0.39
Inter – day (n = 3)	0.32

Table 2: Summary of Method 2, 3, and 4

Parameters	Method 2	Method 3	Method4
Linearity Range (µg/ml)	12 - 84	12 - 84	12 - 84
Correlation coefficient	0.999	0.999	0.999
LOD(µg)	0.18	0.18	0.17
LOQ (µg)	0.16	0.55	0.53
Ruggedness(% RSD)			
Analyst I (n = 6)	0.51	0.42	0.11
Analyst II (n = 6)	0.65	0.33	0.09
Precision (% RSD)			
Repeatability (n = 6)	0.43	0.24	0.08
Intra – day (n = 3)	0.59	0.31	35.68
Inter – day (n = 3)	0.97	0.34	0.24

CONCLUSION:

Four methods RP – HPLC and UV – Spectrophotometry (Zero Order – AUC, First Order Derivative using Amplitude and First Order Derivative using AUC) have been developed for determination of Midodrine Hydrochloride in bulk and tablets. RP – HPLC method are found to be accurate, precise, rugged and sensitive**.** All three UV – Spectrophotometry methods are simple, accurate, precise and least calculations are involved for estimations of concentrations of drug. No interferences of the excipients was observed in any one of the methods during tablet assay. All the developed methods can be used for routine analysis of Midodrine Hydrochloride in pharmaceutical formulation.

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