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Development and Validation of Stability Indicating RP-HPLC Method of Analysis of Manidipine Dihydrochloride

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Abstract: A stability indicating isocratic, rapid high performance liquid chromatographic method was developed and validated for its determination. The determination was performed on a Kromasil ODS C_{18} column (100 × 4.6 mm, 5µ) column with mobile phase consist of ACN - Water (85:15) at a flow rate 1 ml min^{-1} at UV detection wavelength of 229.36 nm. Manidipine Dihydrochloride was found to degrade in acidic and alkaline stress conditions. The drug was stable to Hydrogen peroxide and dry heat. The validation studies were carried out fulfilling ICH requirements. The procedure was found to be linear, precise, and accurate.
Keywords: Manidipine Dihydrochloride; HPLC; ACN; Isocratic; Stability Indicating Method; ICH.

Introduction

Manidipine Dihydrochloride (MAN) is used for antihypertension. It is chemically 1 4 –dihydro-2,6-dimethyl-4(3-nitrophenyl)-3,5-pyridine dicarboxylic acid 2-[4-(diphenyl methyl)-1-piperazinyl] ethyl methyl ester hydrochloride [1]. A literature survery revealed revealed a spectrophotometric [2] and few high performance thin layer chromatographic method (HPTLC) for Manidipine Dihydrochloride determination in human biological fluids [3-5]. However no high performance liquid chromatographic methods were found for Manidipine determination in bulk drug and formulations as a stability indicating assay method.

The International Conference on Harmonization (ICH) guideline entitled "*Stability testing of new drug substances and products*" requires testing to be conducted to assess the inherent stability of the active substances [6]. Test of susceptibility to oxidation, hydrolysis and photolytic degradation are required. An ideal stability indicating method is one that quantifies the drug and resolves its degradation products [7]. HPLC is becoming a routine analytical technique because of advantages [8-11] which include the small amount of mobile phase required, the speed of the method and the possibility of analysis of several samples simultaneously unlike HPLC. It thus reduces analysis time and cost per analysis.

The objective of this work was to develop and validate the accurate, specific, precise, repeatable and stability indicating method for determination of MAN in bulk in the presence of their degradation products.

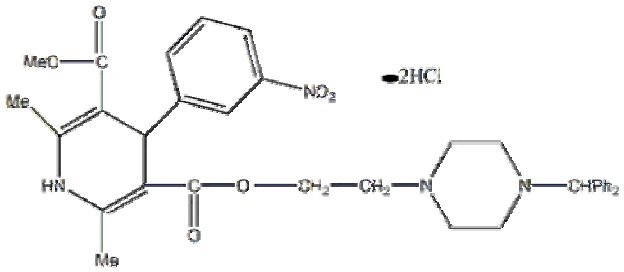

Figure 1: Structure of Manidipine Dihydrochloride

A new method for the HPLC determination of Manidipine Dihydrochloride is described in this paper. The method is substantially simpler, faster and more sensitive.

Experimental

Apparatus

A HPLC (Perkin Elmer Binary LC Pump 200B/ 250) equipped with an inbuilt solvent degasser, Series 200 Pump, Series 200 UV/VIS detector and Kromasil ODS C_{18} column was used with Total Chrome Navigator Software.

Reagent and Materials

HPLC grade Acetonitrile (Sigma Aldrich), AR grade O-Phosphoric acid (Spectrochem Pvt. Ltd), GR grade Hydrochloric acid (Merck Ltd.), GR grade Sodium hydroxide (Merck Ltd.) and distilled water filtered through a 0.45 µm filter (millipore) were used.

Diluent solution

Prepared by mixing with acetonitrile.

Solvent system

The solvent system employed for chromatography consisted by ACN: Water (85:15).

Manidipine Dihydrochloride and its preparation

Pharmaceutical grade Manidipine Dihydrochloride was kindly provided by Pharmten Chemicals Ltd., China.

Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reversed-phase Kromasil ODS C_{18} column (100 × 4.6 mm, 5µ) using a mobile phase consisting of ACN: Water (85:15) at a flow rate 1 ml min^{-1}. The detector wavelength was set at 229.36 nm as determined by Perkin Elmer Lambda 25 UV/VIS spectrometer.

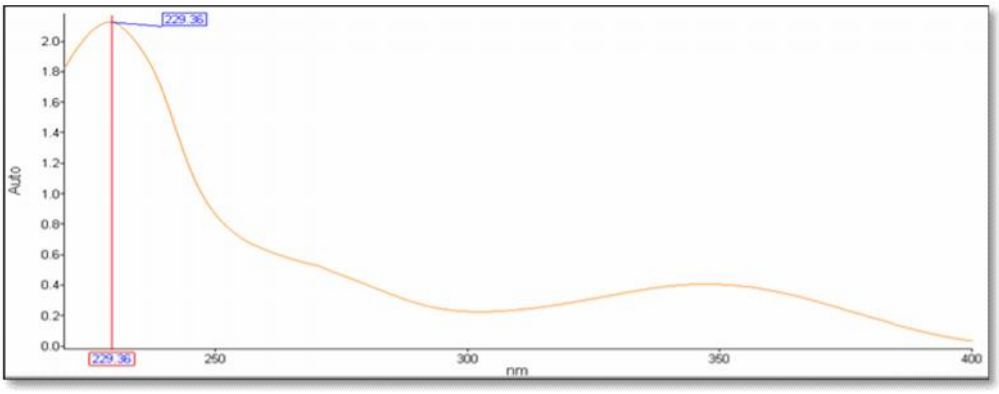

Figure 2: λ_{max} of Manidipine Dihydrochloride by Perkin Elmer Lambda 25UV/VIS Spectrometer

Validation

Linearity

Six serial dilutions were prepared in concentration range from 20 µg mL^{-1} to 100 µg mL^{-1}. A volume of 20 µl from each concentration of the solution was injected and chromatograms were recorded; three independent determinations were performed at each concentration.

Accuracy

To ensure the accuracy of the analytical method, the recovery studies were carried out. Known amount of Manidipine Dihydrochloride was added to a pre quantified sample solution and the amounts of Manidipine Dihydrochloride was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Manidipine Dihydrochloride was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve Accuracy was evaluated at three different concentrations equivalent to 60, 80 and 100 % of the active ingredient by calculating the recovery of Manidipine Dihydrochloride with %RSD.

Precision

The within-day precision of the method was determined for both peak area and retention time by repeat analysis (three identical injections) at three concentration levels. The between day precision was established by performing the analysis over a 5-day period on solution prepared freshly on each day.

Repeatability

Repeatability is the result of the method operating over short time interval (within a day) under the same conditions. The peak area of 20 µg ml^{-1} drug solution was analysed six times on the same day. The %RSD was calculated for the resultant peak area and retention time.

Degradation studies

Acid Hydrolysis

10 mg of Manidipine Dihydrochloride was weighed accurately and transferred to 10 ml volumetric flask and dissolved in 1 N HCl solution. Immediately after making up the volume, the flask was kept at 60°C in hot air oven; 1 ml sample was taken at 5 hrs. and the sample was neutralized with 1 N NaOH solution and diluted to 50 ml with solvent system and loaded into HPLC system.

Alkali Hydrolysis

10 mg of Manidipine Dihydrochloride was weighed accurately and transferred to 10 ml volumetric flask and dissolved in 1 N NaOH solution. Immediately after making up the volume, the flask was kept at 60°C in hot air oven; 1 ml sample was taken at 5 hrs. and the sample was neutralized with 1 N HCl solution and diluted to 50 ml with solvent system and loaded into HPLC system.

Oxidative Degradation

10 mg of Manidipine Dihydrochloride was weighed accurately and transferred to 10 ml volumetric flask and dissolved in 30 % v/v H_2O_2 solution. Immediately after making up the volume, the flask was kept at 60°C in hot air oven; 1 ml sample was taken at 5 hrs. and the sample was diluted to 50 ml with solvent system and loaded into HPLC system.

Thermal Degradation

Powdered API was kept in a hot air oven at 80°C for 4 hrs.

Photo Degradation

Powdered API was kept under direct sunlight for 4 hrs.

Result and Discussion

Method Development

The method utilising Methanol: Water as mobile phase yielded disturbed base line and broad peak, whereas with Methanol: Buffer pH 6.5 tailing was observed. Procedure utilising ACN: Water (60:40) as mobile phase also yielded tailing whereas with ACN: Water (85:15) yielded sharp peak.

During the development of the method, a number of variations were tested. The pH, buffer concentration, ACN concentration and flow rate were chosen to give a symmetric peak with good resolution. With a mobile phase ACN: Water (85:15) well resolved symmetric peak was obtained.

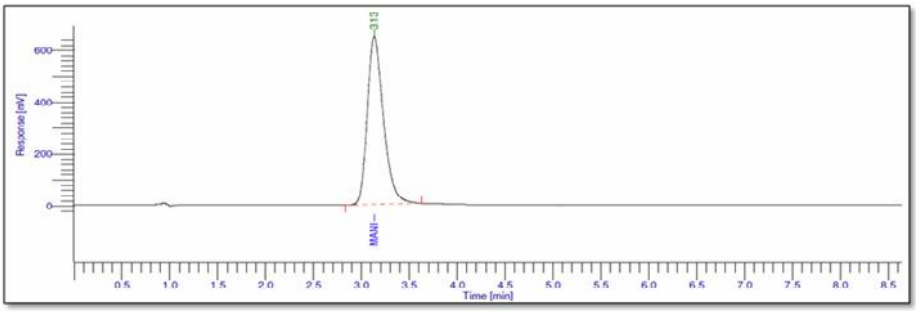

Figure 3: Chromatogram of Manidipine Dihydrochloride

Linearity

A linear calibration graph (y = 77672x + 151482; where y and x are peak area and concentration, respectively) was obtained over five concentrations 20, 40, 60, 80, 100 µg/ml. Correlation coefficient was found to be 0.9953.

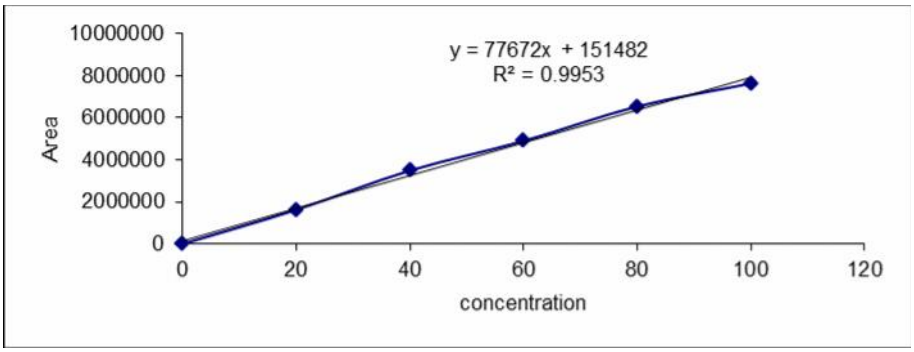

Figure 4: Linearity curve

Accuracy

The accuracy of method was calculated at three concentrations such as 60, 80, and 100 µg mL^{-1} in triplicate. The recoveries at three different concentrations were found to be within the range of 98 to 102% as per ICH guidelines. Mean % recovery (mean ± SD) was found to be 99.46 ± 0**.31 (Table 1).**

Precision

The low RSD **values indicate the ruggedness of the method (Table 2).**

Repeatability (Table 3)

Degradation study

During the study it was observed that upon treatment of Manidipine with acid (1 N HCl), base (1 N NaOH), and hydrogen peroxide (30%) the degradation was observed in base and acid, whereas no degradation was observed with hydrogen peroxide. Following table indicated the extent of degradation of Manidipine Dihydrochloride

under various stress conditions. Following Figure shows the degradation chromatogram of forced degraded samples. Further it is important to note that from the degradation chromatograms, it is evident that although the degrade peaks are observed, under the applied stress conditions like base, heat, acid and photo degradation states. The drug is stable under oxidative degradation condition (**Table 4).**

Table 1: Recovery study (n = 3)

Amount added ($\mu g\ ml^{-1}$)	Amount recovered ($\mu g\ ml^{-1}$)	% Recovered
60	59.87	99.78
80	79.56	99.45
100	99.16	99.16

Table 2: Precision study (n = 3)

Concentration	% RSD	
	Interday	Intraday
20 $\mu g\ ml^{-1}$	0.64	0.35
40 $\mu g\ ml^{-1}$	0.68	0.64
60 $\mu g\ ml^{-1}$	0.15	0.52

Table 3: Repeatability study (n = 6)

Concentration	% RSD[a]	%RSD[b]
20 $\mu g\ ml^{-1}$	0.45	0.75

[a] Based on peak area
[b] Based on retention time

Table 4: Degradation Study at different conditions

Degradation Conditions		Area	% Degradation
Standard	-	7575939.30	-
Acid	1N HCl(60°C; 5 hrs.)	5598619.10	26.1
Alkali	1N NaOH(60°C; 5 hrs.)	5916808.60	21.9
Oxidative	30 % v/v H_2O_2(60°C; 5 hrs.)	7363812.99	2.8
Photolytic	Direct sun light(4 hrs.)	6772889.70	10.6
Thermal	Hot Air Oven(80 °C; 4 hrs.)	7272901.73	4.0

Acid Hydrolysis

Initially 1 N HCl was used for the degradation of manidipine dihydrochloride but it was found that the drug was not stable and there was slight degradation.

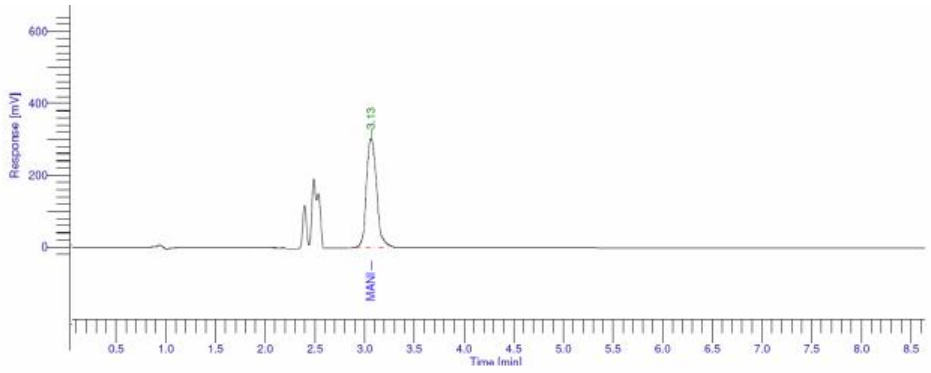

Figure 5: Chromatogram of Manidipine Dihydrochloride in 1 N HCl at 60°C

Alkali Hydrolysis

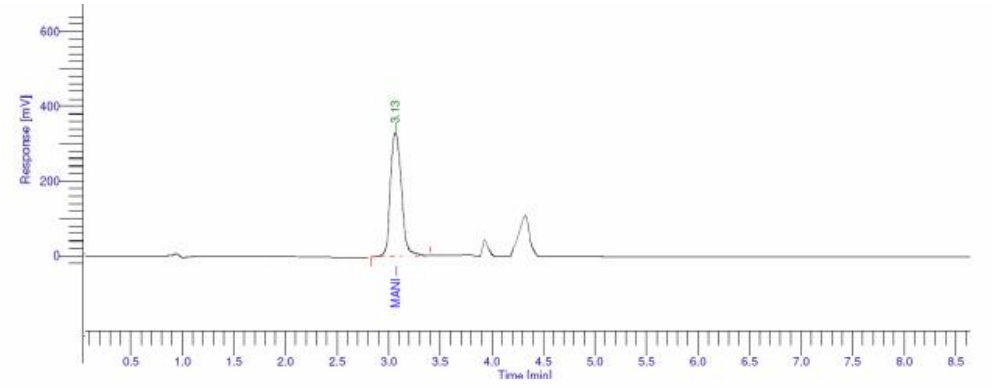

Figure 6: Chromatogram of Manidipine Dihydrochloride in 1 N NaOH at 60°C

Oxidative Degradation

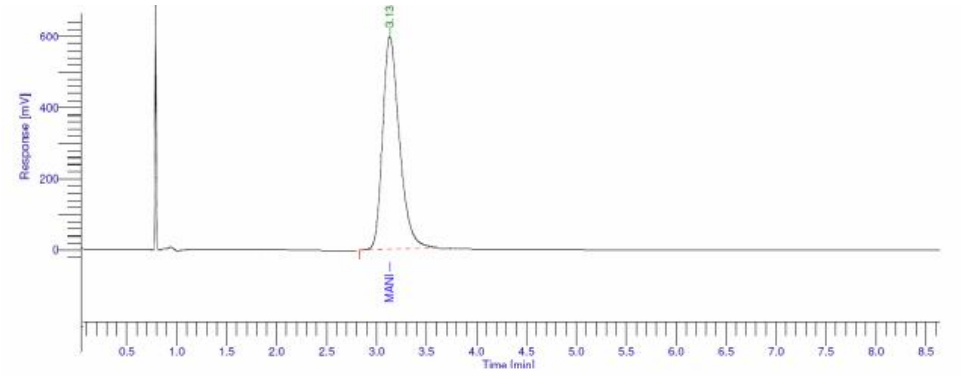

Figure 7: Chromatogram of Manidipine Dihydrochloride in 30% H_2O_2 at 60°C

Photo Degradation

Powdered API was kept under direct sunlight for 4 hrs. From degradation chromatogram, it was found that drug was sensitive to light.

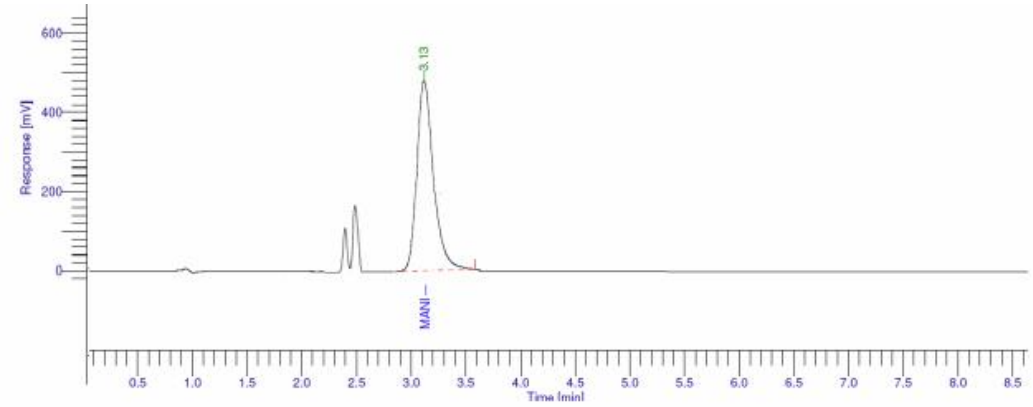

Figure 8: Chromatogram of Manidipine Dihydrochloride exposure to sun light

Thermal Degradation

There was no degradation observed at 80°C for 4 hrs. It indicated that the drug is stable to heat.

Conclusion

The developed HPLC method is precise, specific, accurate and stability indicating. Statistical analysis proved the method is repeatable and selective for the analysis of Manidipine Dihydrochloride as bulk drug. The method can be used to determine the purity of the drug obtained from different sources by detecting related impurities. Because the method separates the drug from its degradation products, it can be used as stability indicating.

Acknowledgement

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