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Stability indicating HPTLC method for quantitative estimation of manidipine sihydrochloride API

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ABSTRACT

A simple, selective, precise and stability indicating high performance thin layer chromatographic method has been established and validated for analysis of manidipine hydrochloride in bulk. The compound was analyzed on aluminium backed silica gel 60 F_{254} plates with methanol:water, 8.5:1.5 (v:v) as mobile phase. The system was found to give compact spots for manidipine dihydrochloride (R_F=0.75). Densitometric analysis was performed at 230 nm. Regression analysis data for the calibration plot indicated good linear relationships between response and concentration over the range of 500-3000 ng/spot. The correlation coefficient, r^2 was 0.998. The values of slope and intercept of the calibration plot were 2785.5 and 62.314, respectively. The method was validated for precision, recovery and robustness. The limits of detection and quantification were 20 and 50 ng, respectively. Manidipine dihydrochloride was subjected to acid, base, peroxide and sunlight induced degradation. In stability test the drug was susceptible to acid and base hydrolysis, oxidation and photodegradation. Statistical analysis proved that the method is repeatable, selective and accurate for manidipine. Because the method could effectively separate the drug from their degradation products, it can be used as a stability indicating method.

1. Introduction

Manidipine dihydrochloride (MAN) is used for anti hypertension. 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 survey revealed a spectrophotometric [2] and few high performance liquid chromatographic methods for manidipine determination in human biological fluids [3-5]. However no high performance thin layer chromatographic method (HPTLC) was found for manidipine determination in bulk drug and formulations as a stability indicating assay method.

The International Conference on Hormonization (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]. HPTLC 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.

2. Experimental

2.1. Instrumentation

Quantitative thin layer chromatographic (TLC) analysis was performed on a Camag Linomat V automated sample applicator with TLC Scanner III. The system was equipped with data acquisition and processing software.

2.2. Materials

Manidipine reference standard was obtained as gift sample from Torrent Laboratories, Gujarat. Methanol was obtained from Merck. Water was prepared in the laboratory using Milli–Q system. Due to the non-availability of the commercial formulation in the local market, synthetic mixture prepared in the laboratory was used for analysis.

2.3. Chromatography

Chromatography was performed on 10 cm × 10 cm aluminium backed plates coated with 0.2 mm layers of silica gel 60 F_{254} (Merck, Germany). Samples were applied to the plates as bands 6 mm wide and 14 mm apart by use of a Camag (Muttenz, Switzerland) Linomat V applicator fitted with a Camag microlitre syringe. The rate of sample application was constant at 150 nL/s. Linear ascending developments of the plates to a distance of 80 mm was performed with methanol:water, 8.5:1.5 (v:v) as mobile phase in a twin trough glass chamber previously saturated with mobile phase vapour

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for 15 min at room temperature. After development the plate was scanned 230 nm by means of a Camag TLC scanner in absorbance mode, using the deuterium lamp. The slit dimensions were 4 mm × 0.3 mm and the scanning speed was 20 mm/s.

2.4. Calibration plot for manidipine dihydrochloride

A stock solution of manidipine (1000 µg/mL) was prepared in methanol. Different volumes of the stock solution (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 µL, equivalent to 500, 1000, 1500, 2000, 2500 and 3000 ng of MAN) were applied in duplicate to a TLC plate. After development, peak height and peak area data and drug concentration data were treated by linear least square regression to determine linearity.

2.5. Method validation

2.5.1. Precision

In accordance with ICH recommendations precision was determined at two levels, i.e. repeatability and intermediate precision. Repeatability of sample application was determined as intraday variation whereas intermediate precision was determined by measuring inter-day variation for triplicate analysis of MAN at two different concentrations (1000 and 2000 ng/spot).

2.5.2. Limit of detection and limit of quantification

MAN at concentrations in the lower part of the linear range of the calibration plot was used to determine limit of detection (LOD) and limit of quantification (LOQ). They were determined from the slope of the calibration plot and standard deviation (S.D.) of the blank sample by use of the equations

$$LOD = 3.3 \times S.D./S \text{ and } LOQ = 10 \times S.D./S \quad (1)$$

Where S.D. is the standard deviation of the blank sample and S is the slope of the calibration plot.

2.5.3. Recovery

Preanalyzed samples were spiked with 50, 100 and 150% MAN standards and then analyzed in triplicate to check recovery from the synthetic mixture at different levels.

2.5.4. Analysis of synthetic mixture

Due to the non-availability of formulations in the local pharmacies, a synthetic mixture was prepared by suitable mixing of 100 mg of MAN with different ratios of excipients like magnesium stearate, talc, starch, lactose and a powder equivalent to 25 mg was weighed. The drug was extracted from the powder with methanol. To ensure complete extraction of the drug it was sonicated for 20 min. The volume was then made up to 25 mL with methanol. Filtered and 1, 2 and 3 µL (1000, 2000 and 3000 ng/spot) of the sample solution were applied on the TLC plate followed by development and scanning as described above. The analysis was repeated in triplicate.

2.5.5. Forced degradation of MAN

2.5.5.1. Acid and base induced degradation

Manidipine dihydrochloride (50 mg) was separately dissolved in methanol and 40 mL of 0.1 M HCl and 0.1 M NaOH. These solutions were heated under reflux for 12 hr and 48 hr at 40 °C, respectively, and then applied on a TLC plate (1000 ng/spot). Chromatography was performed as described above.

2.5.5.2. Hydrogen peroxide induced degradation

Hydrogen peroxide (30% (v:v), 20 mL) was added to separate solution of MAN (25 mg dissolved in 5 mL of methanol). The resulting solution was applied to TLC plate in triplicate and chromatography was performed as described above.

2.5.5.3. Photochemical degradation

MAN (50 mg) powder as such and powder (50 mg) dissolved in 50 mL methanol were exposed to direct sunlight for 24 hrs. The solutions obtained were applied on TLC plates and chromatography was performed as described above.

3. Results and discussion

3.1. Chromatographic conditions

To develop a precise, linear, specific and suitable stability indicating TLC method for analysis of manidipine, different densitogramic conditions were applied. Among the different mobile phases employed the mobile phase consisted of Methanol: water (8.5:1.5; v:v) was found to be suitable for analysis of manidipine dihydrochloride. UV detection of 230 nm was found to be best for analysis.

3.2. Forced degradation studies

Bakshi and Singh [7] suggested a target degradation of 20-80 % for establishing stability indicating nature of the assay method, even as the intermediate degradation products should not interfere with any stage of drug analysis. In the present study, the conditions used for forced degradation are in the range of producing 20-80 % target degradation; degradation of manidipine dihydrochloride could be achieved only after prolonged duration.

During the study it was observed that upon treatment of manidipine with base (0.1 M NaOH), acid (0.1 M HCl) and hydrogen peroxide (30%) the degradation was observed in base and acid, whereas no degradation was observed with hydrogen peroxide. Table 1 indicated the extent of degradation of manidipine under various stress conditions. Figure 1a-e shows the densitograms of forced degraded samples. Further it is important to note that from the densitograms (Figure 1a-e), 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 1. Results of force degradation studies of manidipine dihydrochloride API.

Stress Condition / Duration	% Degradation
Alkaline degradation (0.1 N NaOH, 40 °C, 12 h)	89.93
Acid degradation (0.1 N HCl, 40 °C, 48 h)	91.10
Oxidative degradation (3% H_2O_2, 25 °C, 48 h)	0.94
Thermal degradation (Solid sample, 105 °C, 48 h)	24.38
Thermal degradation (Solution, 80 °C, 48 h)	90.57
Photo degradation (Solid sample, 48 h, sunlight)	27.28
Photo degradation (solution, 48 h, sunlight)	89.27

3.3. Linearity

The calibration curve showed good linearity in the range of 0.5-3.0 µg/spot for manidipine dihydrochloride with correlation co-efficient (r) of 0.9983 (Figure 2). A typical calibration curve has the regression equation of $y = 2785.5x - 62.314$ for manidipine.

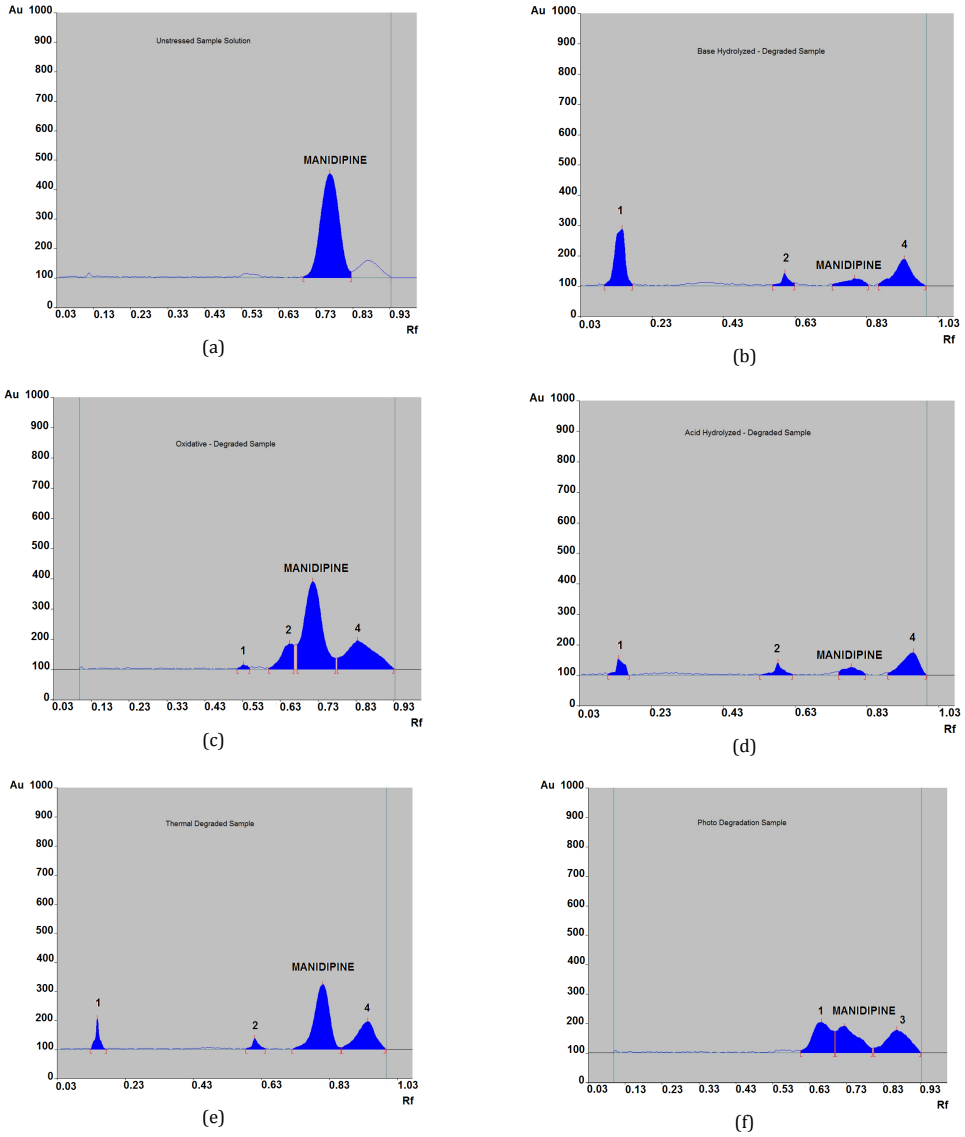

Figure 1. Densitograms of (a) Unstressed sample solution, (b) Base hydrolyzed-degraded sample, (c) Oxidative-degraded sample, (d) Acid hydrolyzed degraded sample, (e) Thermal degraded sample, (f) Photo-degradation sample.

3.4. Precision

The results of method precision (% RSD = 1.014) are found within the prescribed limit of ICH guidelines (% RSD < 2% of method precision).

3.5. Intra-assay and inter-assay

The intra and inter day variation of the method was carried out and the high values of mean assay and low values of standard deviation (SD) and the relative standard deviation (% RSD) (<2%) within a day and day to day variations for manidipine revealed that the proposed method is precise (Table 2 and 3).

Table 2. Intra-assay precision data of proposed HPTLC method (Method Ruggedness).

	Mean (%)	SD	% RSD
Assay-1	99.78	0.22	0.22
Assay-2	98.22	0.28	0.28
Intra assay	99.00	0.25	0.25

SD: Standard deviation; RSD: the relative standard deviation.

Table 3. Inter-assay precision data of proposed HPTLC method.

	Mean (%)	SD	% RSD
Assay-1	99.49	0.20	0.27
Assay-2	100.74	0.24	0.32
Inter assay	100.11	0.22	0.29

SD: Standard deviation; RSD: the relative standard deviation.

3.6. Limit of detection and limit of quantification

The minimum concentration levels at which the analyte can be reliable detected (LOD) and quantified (LOQ) were found to be 0.020 µg/spot and 0.050 µg/spot, respectively.

3.7. Specificity and stability in analytical solution

The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that manidipine was stable in solution form up to 24 h at 25 °C and 48 h at 25 °C when protected from light (kept in dark). The results of linearity, precision, inter and intra-day assays, LOD, LOQ and specificity and stability in analytical solution established the validation of the developed TLC assay for the analysis of manidipine.

4. Conclusion

The developed HPTLC method is precise, specific, accurate and stability indicating. Statistical analysis proved the method is repeatable and selective for the analysis of manidipine as bulk drug. The method can be used to determine the purity of the drug obtained from different sources by detecting related impurities. It may be extended to determination of the degradation kinetics of manidipine in biological fluids. Because the method separates the drug from its degradation products, it can be used as stability indicating.

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