DETERMINATION OF MANIDIPINE HYDROCHLORIDE IN TABLETS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT: A High Performance Liquid Chromatography (HPLC) method was developed to determination Manidipine hydrochloride in tablets. The system comprises of a Symmetry C-18 (4.6 x 150 mm, 3.5 µm i.d.) as stationary phase, a 45 : 55 mixture of ammonium formate buffer (25 mM, pH 3.1) as mobile phase, flow rate 0.7 ml/min, column temperature at 30oC and detection wavelength at 230 nm. Furthermore, the method was studied stress degradation which carried out under the conditions of hydrolysis (1N NaOH and 1N HCl), oxidation (30% H2O2), photolysis (UV 254 nm) and thermal degradation (60oC) were investigated. Validation of the optimum condition was performed on commercially Manidipine hydrochloride tablet formulations. Validation on the method was assessed from the specificity, linearity and range, precision, accuracy, limit of detection and quantification, stability of solution and system suitability. The linear ranges of 50 - 150 µg/ml was found for Manidipine hydrochloride. The system and method precision calculated from the relative standard deviation was less than 2%. The percentage recovery on accuracy was 99.42%, the limit of detection and quantification were 0.14 and 0.43 µg/ml, respectively. The purposed method was successfully applied to the direct determination of Manidipine hydrochloride in tablet formulations. The results were statistically compared with those of the reference method (Japanese Pharmacopoeia, 15th edition, Supplement I).

Keywords: Manidipine, 1.4-Dihydropyridine derivatives, HPLC, Validation

INTRODUCTION

Manidipine hydrochloride (MND), 2-[4-(diphenylmethyl)-1-piperazinyl]-ethylmethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate dihydrochloride (Figure 1), is a third generation drug of the well-known pharmacological active compound series classified as 1,4-dihydropyridine calcium channel blockers. This drug is used in the treatment of angina pectoris, hypertension and congestive heart failure [1, 2]. A literature survey revealed only a few spectrophotometric methods [3, 4] and HPLC methods [5, 6] reported for the determination of MND in bulk and tablets and biological fluids, respectively. The compendial methods for the assay of MND in bulk and Tablets are only available in the Japanese Pharmacopoeia, Supplement 1 [7]. Attempts were hence made to develop a simple, precise and stability indicating HPLC assay method for analysis of MND.

MATERIALS AND METHODS

Materials

Manidipine hydrochloride working standard was produced from Xiamen Shinbon Chemicals (China) and tablet (Madiplot) was purchased from Takeda (Japan). Sodium hydroxide was purchased from Mallinckrodt Baker Inc. (Mexico) and hydrochloric acid was produced from Merck (Germany). Hydrogen peroxide was produced from BHD (England). HPLC-grade of acetonitrile and methanol were purchased from Burdick and Jackson (USA). All other chemicals were of an analytical reagent grade.

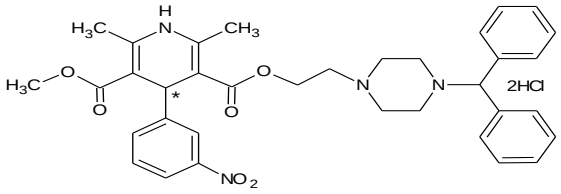

Figure 1 Structure of Manidipine hydrochloride (The stereogenic center is marked with an asterisk)

Instrumentation

The HPLC system was equipped with an LC-10AVP pump, an SPD-10AVP UV-Vis detector, an SIL-10ADVP auto-injector and a DGU-14A degasser module; data were acquired and processed using a CLASS-VP software (all from Shimadzu, Kyoto, Japan). The chromatographic separations were carried out on Symmetry (Waters Corporation, Ireland) C-18 column (250 mm x 4.6 mm i.d., with

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a particle size of 3.5 µm).

Sample and Standard preparation

Sample preparation

Weighed and finely powdered not less than 20 tablets of MND hydrochloride Tablets. Transferred an accurately weighed portion of the powder, equivalent to about 10 mg of MND hydrochloride, to a 100-ml volumetric flask, added 10 ml of methanol and sonicate for 5 minutes. Allowed the solution to cool to room temperature, diluted with diluent to volume, and mixed. Passed a portion of this solution through a filter with a pore size of 0.45 µm, discarding the first 4 ml of the filtrate. Subsequent filtrate obtained was used as the stock sample solution.

Standard preparation

Accurately weighed and transferred 100 mg of manidipine hydrochloride standard to a 100-ml volumetric flask, added 10-ml of methanol and sonicated for 5 minutes. Allowed the solution to cool at room temperature then diluted with methanol to volume and mixed. The solution concentration was 100 µg/ml, kept in a refrigerator at 4oC and protected from light.

Stressed testing study

All stress decomposition studies were performed at an initial drug concentration of 0.1 mg/ml. Acid hydrolysis was performed in 1N HCl at 60 oC for 3 h and basic hydrolysis was carried out in 1N NaOH at 60 oC for 3 h. Oxidation study was carried out in 30% H2O2 at 60 oC for 3 h. For photolysis study, samples were exposed to UV 254 nm for 3 h. The thermal degradation of MND was also studied at 60 oC for 3 h.

Optimization of chromatographic conditions

The reversed-phase HPLC conditions were optimized by varying the buffer pH (2.8, 3.1, 3.4), the buffer concentration (20, 25, 30 mM), the detection wavelength (225, 230, 235 nm) and the column temperature (27, 30, 33 oC).

Analytical method validation

The method performance characteristics examined according to the ICH guidelines [8] were specificity, linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and stability of standard and sample solutions. The system suitability study was also carried out.

Identification of degradants

The degradants were analyzed by LC-MS with the same chromatographic conditions as mentioned in the reversed-phase HPLC. The mass spectra of stressed samples were recorded in the range 100 – 1000 [M-zH+], using the Electron Spray Ionization (ESI) method.

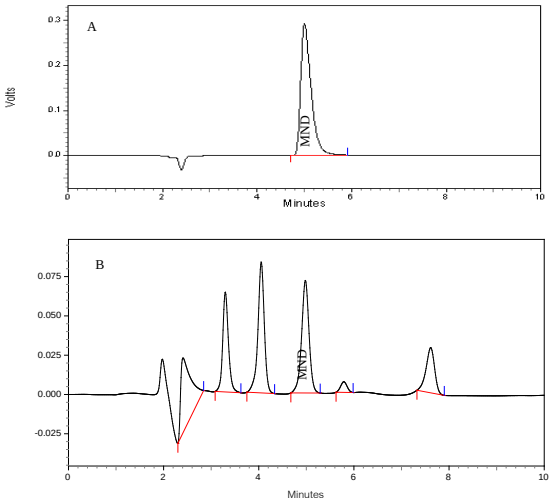

Figure 2 Chromatogram of Manidipine in normal sample (A) and in basic stressed sample (B)

RESULTS AND DISCUSSION

The optimum reversed-phase, C18, HPLC condition for the determination of MND was obtained with 25 mM of ammonium formate buffer (pH 3.1)-acetonitrile (45:55, v/v), as mobile phase, the detection wavelength of UV 230 nm, and the column temperature at 30 oC. A chromatogram of the stress sample obtained from analysis performed under these optimum conditions is shown in Figure 2 with a retention time of MND of 4.9 min.

The plot of concentration versus peak area of MND showed good linearity in the 50-150 µg/ml with the coefficient of determination (r2) of 0.9992. The precision of system and method expressed as relative standard deviation was less than 2%. The method accuracy of 99.42% was reported as the average percentage recovery of MND in spiked samples. The limit of detection and quantification were 0.14 and 0.43 µg/ml, respectively.

The peak result of MND in normal sample solution was 5.1 min. On stressed degradation study of MND, four degradation peaks (at 3.3, 4.0, 5.7 and 7.5 min) were observed in basic stress sample, but not in acid stress sample. Degradant (at 5.6 min) was also detected in photolysis, thermal degradation and oxidized samples as shown in Figure 2.

The structural characteristic is the 1,4-DHP moiety exhibiting phenyl substitution in position 4. The vascular selectivity of 1,4-DHP is apparently coupled to the chemistry of the substitution in 2-position, the phenyl substituents in 4-position of the DHP-ring with a nitro group which makes it very light sensitive. The main photodegradation product, the nitrophenylpyridine derivative, is a result of oxidation of the DHP ring which leads to aromatization of the DHP system. MND, nitrophenylpyridine derivative, and nitrozophenylpyridine derivative were characterized by the retention times at 4.9, 5.7 and 4.0 min,

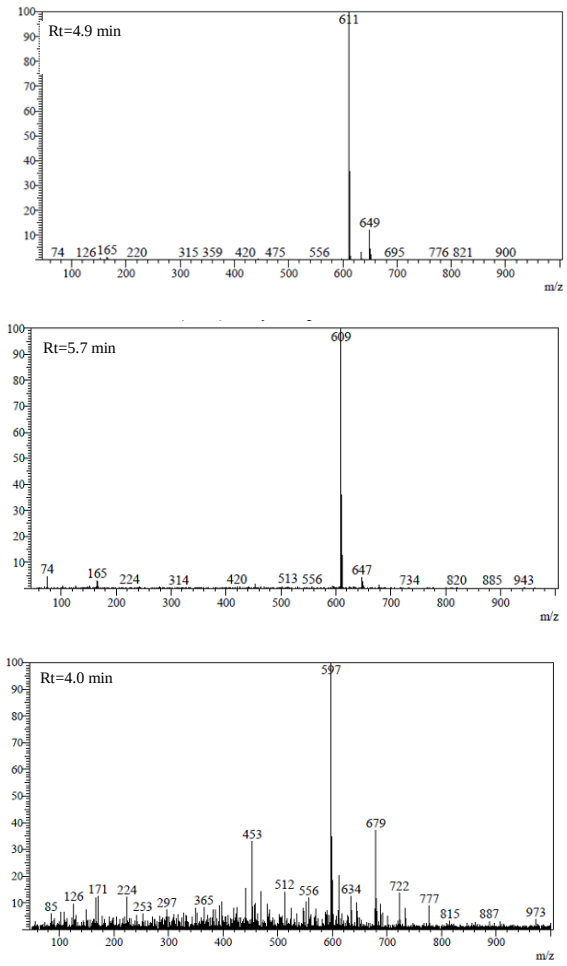

Figure 3 Mass spectra of Manidipine and products of its photodegradation (1) manidipine (Rt= 4.9 min); (2) photoproduct I (Rt=5.7min) - nitrophenylpyridine derivative and (3) photoproduct II (Rt=4.0min) – nitrozophenylpyridine derivative.

respectively. The molecular ions corresponding to MND, nitrophenylpyridine derivative, and nitrozophenylpyridine derivative were respectively 611, 609, and 597 as shown in Figure 3.

CONCLUSION

An HPLC method has been developed and validated for the analysis of MND in tablet formulations. The method is simple, rapid, accurate and precise. The results of stress testing study reveal that the method is selective and stability-indicating. The proposed method can be applied to the analysis of samples obtained during accelerated stability experiments.

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