VALIDATION OF STABILITY INDICATING ULTRA-FAST LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF ATENOLOL and NIFEDIPINE IN BOTH BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: The study depicts improvement of ensuing validation of a stability indicating technique for the simultaneous estimation of Atenolol and Nifedipine using Ultra-fast liquid chromatographic method (UFLC).

Methods: The analysis is performed on Phenomenex Kinetix C18 (150 × 4.6 mm, 5μm) column using methanol and 0.1% ortho-phosphoric acids (75:25 v/v) as mobile phase with a flow rate of 1.3 ml/min. The eluents were checked with PDA detector at 237 nm.

Results: In this optimized conditions Atenolol and Nifedipine elutes at a retention time of 2.79 and 4.50 min respectively individually the considered optimized condition is having linearity in the range from 10 to 50µg/ml of Atenolol and 4-20µg/ml of Nifedipine. The method was validated by following the ICH guidelines and their combination drug yield was exposed to acid and base stress, thermal stress, photolytic stress, hydrolytic stress, and oxidative stress conditions. All samples were studied by the given optimized method. In this Calibration curves were linear over studies ranges with correlation coefficient found between the ranges of 0.99 to 1.00.

Conclusion: The proposed method was found to be accurate, precise, and specific and suitable for determination of both the drugs.

Keywords: Atenolol, ICH guidelines, Nifedipine, Stability indicating studies, UFLC

INTRODUCTION

Atenolol, 4-[2-hydroxy-3-[(1-methyl ethyl) amino] propoxy]-benzeneacetamide [1] (fig. 1) is a cardio-selective β1-adrenergic receptor blocking agent recommended for the treatment of hypertension, angina pectoris, and cardiac arrhythmias. It is a Beta blocker that intrudes with binding to the receptor of epinephrine and different stress hormones and decreases the impacts of these hormones. Beta blockers are especially utilized for the management of cardiovascular arrhythmias, shielding the heart from second attack (myocardial infarction) after a first heart attack and hypertension [2].

Nifedipine is dimethyl-4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine 3,5dicarboxylate [3] (fig. 2) Nifedipine is a calcium channel blocking agent. The principal activity of calcium channel blockers incorporate dilatation of coronary and fringe coronary and peripheral arteries and arterioles, negative in tropic activity, decrease the heart rate, and decelerate the atrioventricular (AV) conduction. It restrains the Trans layer influx of calcium ions into vascular smooth muscle and cardiovascular muscle. Nifedipine restrains calcium ions influx across cell membranes specifically, with a more impact on vascular smooth muscle compared to cardiac muscle cells[4] Combined use of Atenolol with Nifedipine decreases the properties of cardiac muscles especially in patients with ventricular or conduction abnormalities [5-7].

The proposed technique was optimized and validated as per International Conference on Harmonization (ICH) guidelines. [8-10]. The aim of the present work is to develop a simple, fast, precise and accurate reversed-phase chromatographic method together with stability indicating studies for the both mix drugs Atenolol and Nifedipine in bulk and its pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and reagents

The HPLC grade methanol is acquired from Merck Pvt Ltd, Mumbai. The chemicals utilized are of analytical grade (AR grade) like orthophosphoric acid obtained from Loba Chemie, Mumbai.

Instrumentation

The SHIMADZU, UFLC with PDA detector and LC solution software was utilized in the current research work. The separation was accomplished using C18 column. The mobile phase contains of 0.1% orthophosphoric acid in water and methanol (75:25 v/v). The mobile phase was filtered before use through membrane filters (0.45μ). The upgraded chromatographic conditions were mentioned in given table 1.

Table 1: Chromatographic conditions

| Parameter | Condition |
|-----------|-----------|
| Mobile Phase | 0.1% orthophosphoric acid in water and methanol (75:25 v/v) |
| Column | C18 |
| Flow Rate | 1.3 ml/min |
| Detector | PDA at 237 nm |
| Temperature | 25°C |

Fig. 1: Chemical structure of atenolol

Fig. 2: Chemical structure of nifedipine
Table 1: Optimized chromatographic conditions

| Parameter            | Specification                                                                 |
|----------------------|-------------------------------------------------------------------------------|
| Column               | C18 (150 × 4.6 mm, 5μm) Phenomenex Kinetex                                    |
| Flow rate            | 1.3 ml/min                                                                    |
| Run time             | 10 min                                                                        |
| Wavelength           | 238 nm                                                                        |
| Injection Volume     | 20μL                                                                          |
| Detector             | PDA Detector                                                                  |
| Elution              | Isocratic                                                                     |
| Mobile Phase         | Methanol and 0.1 % ortho-phosphoric acid (75:25 v/v)                          |
| Column oven temperature | 25±5 °C                                                                 |

Preparation of mobile phase

The mobile phase is prepared by adding 1 ml of orthophosphoric acid in 1000 ml water (ie; 0.1% orthophosphoric acid in 1000 ml water) and methanol this mobile phase is ultra-sonicated used for 20 min were used in the ratio of 75:25 (v/v).

Preparation of standard solutions

A standard stock solution of Atenolol and Nifedipine was prepared by dissolving 50 mg Atenolol and 20 mg of Nifedipine drugs in 50 ml of methanol made up to the volume by dissolving completely using the methanol to get the standard stock solutions of concentration 1000μg/ml for Atenolol and 400μg/ml Nifedipine.

Preparation of calibration curve

From the standard stock solutions, different aliquots of Atenolol and Nifedipine were pipetted into series of 10 ml volumetric flask from the above stock preparation (1000 μg/ml). HPLC grade methanol was used for making up the volume. 20μl solution was injected to the column and peak areas are measured. The calibration curve was established linear correlations were found between peak scales. Atenolol and Nifedipine concentration are defined my means of regression equation (fig. 3 and fig. 4 respectively). The Beer’s law is observed in the concentration scale of 10-50 μg of Atenolol and Nifedipine 4-20μg/ml Estimation of two drugs was done through PDA detector at 238 nm.

Preparation of sample solution of formulation

Into a dry 50 ml volumetric flask finely grounded and mixed contents of 20 capsules with equivalent weights of 50 mg Atenolol and 20 mg of Nifedipine were taken and ultra-sonicated until the drug dissolved in methanol then made up to the volume. At 238 nm area of each peak was measured. From the peak area, we determine the amount of each drug, Atenolol and Nifedipine respectively present in the pure mixture. Upon further quantitative dilution of this solution with mobile phase, a final concentration of 50 mg/ml of Atenolol and Nifedipine was obtained.

RESULTS AND DISCUSSION

Linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ), robustness is the parameters to be validated for all samples according to the ICH guidelines using above chromatography conditions.
Linearity

Linear calibration curves of both Atenolol and Nifedipine were obtained based on the above chromatographic conditions. The r² for Atenolol and Nifedipine were found to be 0.979 and 0.967 respectively. Between the peaks area of Atenolol and Nifedipine linear correlations were found and are described by using regression equation. Table 2 specifies the results. For system suitability, Atenolol and Nifedipine and the linearity range were found to be 0-50μg/ml and 4-20μg/ml respectively.

Precision

Repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility) are the terms to determine the method precision mentioned below in table 3 and 4.

Accuracy

According to the test procedure triplicates of samples solutions by spiking with the test solutions of Atenolol and Nifedipine 50%, unstressed sample(fig. 8) Here the bulk drug is subjected to acidic stress by adding 1.0 ml of 0.1M HCl (fig. 9) to drug solution and neutralized with 1.0 ml of 0.1M NaOH, at 0 min, 30 min, 1 h, 2 h, 4 h, 8 h, 6 h and 32 h respectively. Similarly, the basic stress studies were performed by adding 1.0 ml of 0.1M NaOH (fig. 10) and neutralized with 1 ml of 0.1M HCl. Thermal studies were performed by heating the sample at 60 °C. In the development phase of a method, robustness should be considered earlier-stated by ICH guideline.

Mobile phase composition, pH, flow rate, temperature, wavelength, and UV studies were also carried out by the sample at UV-Lamp 450C, (fig. 12)

Robustness

A measure of capacity to stay unaffected by small, but deliberate variations in the final method optimized conditions, called robustness for an analytical procedure as per ICH guidelines. The method development with predictable variations in the optimized method parameters is the most significant feature here. In the development phase of a method, robustness should be considered earlier-stated by ICH guideline.

Forced degradation studies

The stress studies were performed on Atenolol and Nifedipindrug at 50µg/ml concentration. unstressed sample(fig. 8) Here the bulk drug is subjected to acidic stress by adding 1.0 ml of 0.1M HQ (fig. 9) to drug solution and neutralized with 1.0 ml of 0.1M NaOH at 0 min, 30 min, 1 h, 2 h, 4 h, 8 h, 6 h and 32 h respectively. Similarly, the basic stress studies were performed by adding 1.0 ml of 0.1 M NaOH(fig. 10) and neutralized with 1 ml of 0.1M HCl. Thermal studies were performed by heating the sample at 60 °C(fig. 11) Oxidation studies were performed on the bulk drug by adding 2 ml of 3% H₂O₂(fig. 12) and UV studies were also carried out by the sample at UV-Lamp 450C.
(fig. 13) respectively. All samples were placed in a different volumetric flask (10 ml) and dissolved in HPLC grade methanol. Chromatographic system injected with final drug concentration for a ssay made with methanol. For all these stability study, the formation of degradable product was confirmed by comparing with the chromatogram of the solution kept under normal unstressed condition. All stressed samples were analysed by optimized UFLC method. The degradation data for Atenolol and Nifedipine was shown in below table 8.

Table 5: Recovery results for atenolol and nifedipine

| Recovery % | Amount of std drug added (µg/ml) | Amount of drug added (µg/ml) | Total amount of drug (µg/ml) | Difference | % Recovery | Mean |
|------------|----------------------------------|-----------------------------|-------------------------------|------------|------------|------|
| 50         | 20                               | 10                          | 30                            | 150652     | 100.36     | 99.19 |
| 100        | 20                               | 20                          | 40                            | 148341     | 98.69      | 98.51 |
| 150        | 20                               | 30                          | 50                            | 150047     | 99.95      | 99.62 |

Table 6: Results of robustness for atenolol

| Condition                          | Tailing  | % RSD | Theoretical plates | %RSD  |
|------------------------------------|----------|-------|--------------------|-------|
| As such condition (optimized method)|          |       |                    |       |
| Mobile phase ratio                 | 70:30    | 0.66  | 4037.3             | 1.48  |
| As such (75:25)                    | 85:15    | 0.28  | 4048.3             | 1.34  |
| % of Ortho-phosphoric acid         | Decreased (-0.2 units) | 1.847 | 1.29 | 4284.9 | 1.50 |
|                                    | Increased (+0.2 units) | 0.98 | 1.87 | 4255.1 | 1.15 |
| Flow rate                          | Decreased (-0.2 ml/min) | 1.020 | 0.89 | 4267.39 | 1.29 |
|                                    | Increased (+0.2 ml/min) | 1.099 | 1.20 | 4250.43 | 1.09 |
| Column temperature                 | Decreased (-5 °C) | 1.267 | 1.32 | 4048.29 | 1.34 |
|                                    | Increased (+5 °C) | 1.183 | 0.83 | 4302.93 | 1.71 |
| Wave length                        | Decreased (1 nm) | 0.545 | 1.37 | 4429.39 | 1.08 |
|                                    | Decreased (2 nm) | 1.288 | 1.60 | 4312.2 | 1.81 |
|                                    | Increased (1 nm) | 1.373 | 1.74 | 4313.22 | 1.83 |
|                                    | Increased (2 nm) | 1.218 | 1.47 | 4292.08 | 1.58 |

Table 7: Results of robustness for nifedipine

| Condition                          | Tailing  | % RSD | Theoretical plates | %RSD  |
|------------------------------------|----------|-------|--------------------|-------|
| As such condition (optimized method)|          |       |                    |       |
| Mobile phase ratio                 | 70:20    | 0.141 | 3732.39            | 1.47  |
| As such (75:25)                    | 85:15    | 1.19  | 3348.30            | 1.28  |
| % of Ortho-phosphoric acid         | Decreased (-0.2 units) | 1.846 | 0.84 | 4946.9 | 1.51 |
|                                    | Increased (+0.2 units) | 1.298 | 1.16 | 4356.74 | 1.42 |
| Flow rate                          | Decreased (-0.2 ml/min) | 1.170 | 1.93 | 4861.39 | 1.84 |
|                                    | Increased (+0.2 ml/min) | 1.249 | 0.97 | 4285.43 | 0.81 |
| Column temperature                 | Decreased (-5 °C) | 1.167 | 0.82 | 4948.29 | 1.59 |
|                                    | Increased (+5 °C) | 1.585 | 1.22 | 4202.93 | 1.82 |
| Wave length                        | Decreased (1 nm) | 0.835 | 1.22 | 4839.39 | 1.39 |
|                                    | Decreased (2 nm) | 1.18  | 1.34 | 3893.92 | 1.92 |
|                                    | Increased (1 nm) | 1.448 | 0.46 | 4839.22 | 1.58 |
|                                    | Increased (2 nm) | 1.78  | 1.93 | 3772.08 | 1.59 |

Fig. 8: Chromatogram of unstressed sample
Fig. 9: Chromatogram of acid hydrolysis

Fig. 10: Chromatogram of base hydrolysis

Fig. 11: Chromatogram of thermal stress

Fig. 12: Chromatogram of peroxide stress
Table 8: Results for recovery studies of atenolol and nifedipine after the stress conditions (% recovery of drug)

| Time  | Drug | UV    | Thermal | 0.1N HCL  | 0.1N NaOH | 3%H₂O₂ |
|-------|------|-------|---------|-----------|-----------|---------|
| 0 Min | Atenolol | 82.24% | 73.11%  | 71.65%    | 72.34%    | 56.47%  |
|       | Nifedipine | 84.23% | 76.76%  | 87.79%    | 89.35%    | 81.34%  |
| 30 Min| Atenolol | 77.34% | 60.76%  | 57.29%    | 61.34%    | 44.19%  |
|       | Nifedipine | 80.34% | 67.31%  | 84.14%    | 87.34%    | 74.34%  |
| 1 h   | Atenolol | 69.32% | 47.86%  | 52.3%     | 54.34%    | 32.47%  |
|       | Nifedipine | 72.43% | 50.16%  | 78.86%    | 80.34%    | 68.23%  |
| 2 h   | Atenolol | 61.73% | 28.66%  | 37.47%    | 42.34%    | 25.19%  |
|       | Nifedipine | 67.34% | 37.14%  | 74.78%    | 78.38%    | 60.87%  |
| 4 h   | Atenolol | 54.22% | 19.81%  | 28.07%    | 30.87%    | 15.47%  |
|       | Nifedipine | 59.34% | 21.69%  | 67.27%    | 70.34%    | 44.34%  |
| 8 h   | Atenolol | 47.62% | 8.89%   | 14.64%    | 13.32%    | 4.43%   |
|       | Nifedipine | 52.23% | 30.15%  | 59.65%    | 57.23%    | 32.62%  |
| 16h   | Atenolol | 39.22% | ---     | ---       | 6.34%     | ---     |
|       | Nifedipine | 43.87% | ---     | ---       | 44.64%    | 22.23%  |
| 32h   | Atenolol | 22.43% | ---     | ---       | ---       | ---     |
|       | Nifedipine | ---   | ---     | ---       | ---       | ---     |

CONCLUSION

A simple, quick, sensitive, reliable, and precise stability indicating UFLC method was developed and validated for the estimation of Atenolol and Nifedipine. The method was observed to be linear, accurate, precise, and turned out to be sensitive, convenient and successful with good resolution for the estimation of Atenolol and Nifedipine in both bulk and pharmaceutical dosage forms in industries and research labs for routine sample analysis.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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