HPLC METHOD DEVELOPMENT AND VALIDATION OF LERCANIDIPINE HCL AND ATENOLOL, CHARACTERIZATION OF ITS DEGRADANTS BY LC-MS/MS

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ABSTRACT

Objective: An assay method was developed and validated for the simultaneous estimation of Lercanidine HCl and atenolol using RP-HPLC.

Methods: An effective chromatographic separation was achieved using Waters Symmetry C18 column of dimensions 150x4.6 mm, 3.5 μm, as a stationary phase. 0.1 percent ortho phosphoric acid and acetonitrile in 50:50 v/v was used as a mobile process with a rate of flow 1 ml/min and UV detection was carried out at 230 nm, respectively. Isocratic chromatography at ambient temperature was performed.

Results: Lercanidine HCl and atenolol were separated by a running time of around 8 min. at 2.925 min. and 6.482 min. Respectively. By injecting the norm six times, device suitability parameters were studied and the outcomes were well under the acceptance criteria. The linearity analysis was performed at levels ranging from 10% to 150% and the R² value was found to be 0.999.

Conclusion: Assay method validation was performed by using the marketed formulation and found to be within the limit. Degradation tests were conducted and the degradants were characterized by using LC-MS/MS.

Keywords: Development, Validation, Lercanidine HCl, Atenolol, LC-MS/MS

INTRODUCTION

Lercanidine is an anti-hypertensive [1, 2] drug and belongs to the calcium channel blocker [3, 4] class of dihydropyridine, which works by relaxing, and by relaxing expanding vessels of blood so that blood can flow around the body more openly it lowers blood pressure and makes it possible for the heart to function more effectively [5]. The medication have less adverse effects, but a comparatively high drug interaction potential. Lercanidine is used to treat hypertension (high blood pressure). Lercanidine, like other dihydropyridine, is contraindicated in patients with unstable angina [6, 7], uncontrolled heart failure [8, 9], immediately after myocardial infarction [10, 11], and in patients with obstruction of outflow of left ventricular. During and in women who have become pregnant may become pregnant, it is also contraindicated because there is a lack of safety evidence as well as in patients with extreme liver and kidney disease, for the unborn.

Atenolol is a beta-blocker drug [12, 13] used mainly for the treatment of elevated blood pressure and chest pain [14, 15] associated with the heart. However, atenolol does not seem to increase survival in patients with elevated blood pressure [16, 17]. Additional applications include migraine prevention [18] and the treatment of some irregular heartbeats [19]. It is taken by mouth or into a vein by injection. Other serious side effects include bronchospasm [20]. Tired feelings, heart failure, dizziness, anxiety and shortness of breath are common side effect. Use during breastfeeding. Is not recommended and alternative medications are used for breast feeding. It functions by blocking the hearts β1-adrenergic receptors, thereby reducing the heart rate and load.

Fig. 1 shows the chemical representations of Lercanidine HCl and Atenolol. This paper proposes a novel sensitive stability-indicating RP-HPLC procedure for the assessment of Lercanidine HCl and Atenolol combination. The process proposed enables the rapid assessment of the Lercanidine HCl and Atenolol in bulk drugs and formulation preparations without sample pre-treatment with high precision and specificity and with no excipient intervention.

MATERIALS AND METHODS

Chemicals
Acetonitrile, orthophosphoric acid and water all are of HPLC grade, were purchased from Merck India Pvt Ltd., Worli, Mumbai, India.
APIs of Lercanidipine HCl, atenolol were purchased from Spectrum Para research solutions Pvt Ltd, Hyderabad.

**Equipment**

**HPLC**

The chromatographic device of Waters quaternary pump alliance e-2695, PDA detector 2998 and chromatographic software Empower-2.0 were used.

**LC-MS/MS**

An HPLC system (waters alliance e2695 model) connected with mass spectrometer QTRAP 5500 triple quadrupole instrument (sciex) was used. By the Empower 2.0 software operation was performed. Working parameters of mass spectrometry after optimization as follows: Ion spray voltage 5500V; temperature source 550 °C; Drying gas- Nitrogen; Pressure 55ps; Drying gas flow stream-5 ml/min; Delustering potential 40V; Entrance potential 45V; Exit potential 15V; Capillary voltage 5500V and Dwell time 1Sec respectively.

**Preparation of buffer**

In 1 lt of HPLC Water, 1 ml of orthophosphoric acid was dissolved and filter through 0.45 µ filter paper.

**Preparation of mobile phase**

Acetonitrile and buffer were mixed in 50:50 v/v ratio and sonicated for 5 min. After that filtered it by using 0.45µm membrane filter paper.

**Diluent**

Mobile phase.

**Preparation of standard solution**

Standard stock solution of Lercanidipine HCl and Atenolol was prepared by appropriately estimating about 10 mg and 50 mg drug in 100 ml volumetric flask. Then the drug was liquified insolvent and filter through a 0.45µ filter. Standard stock solution concentrations of 100μg/ml and 500μg/ml were obtained.

**Preparation of the solution for samples**

Ten lercanidipine HCl and Atenolol tablets were accurately weighed and triturated to get a fine powder. A 10 mg Lercanidipine HCl and 50 mg Atenolol equivalent weight tablet powder was transferred into a 100 ml volumetric flask and dissolved in the diluent. The solution was ultra-sonicated for 10 min and made the volume with diluent. The tablet sample solution was then filtered through 0.45 micron syringe filter and utilized for preparing sample solution for the assay.

**Optimization of chromatographic conditions**

Various combinations of mobile phases were screened with respect to resolution, theoretical plate count, tailing and other system suitability parameters. Finally, the separation was performed with freshly prepared mobile step is composed of Acetonitrile and buffer in 50:50 v/v ratio with a 1 ml/min flow rate. Wavelength of 230 nm with injection volume 10 μl and ambient temperature was maintained during the entire process to obtain a symmetric peak of Lercanidipine HCl and Atenolol.

**Method optimization**

The current study was designed to develop a simple, reliable and rapid analytical RP-HPLC system which can be used to evaluate assay method of current estimation of Lercanidipine and atenolol pharmaceutical and bulk dosages forms. In order to have good results for the assay, the chromatographic conditions were optimized. Different combinations of Lercanidipine and atenolol have been tried to optimize the mobile process. The final working mobile phase was acetonitrile: 0.1 percent OPA at 50:50v/v. Based on its polarity, the mobile phase was selected for each drug. In order to achieve adequate sensitivity for the two smaller proportions of APIs (Lercanidipine and atenolol), the detection was carried out at several wavelengths. Finally, as a detection wavelength, the 230 nm wavelength at which the two drugs showed strong absorbance was chosen. The rate of flow was 1.0 ml/min, which is important as it affects the parameters of peak symmetry. The retention time for Lercanidipine and atenolol was 2.925 min and 6.482 min respectively. The suggested approach is checked in compliance with the ICH guidelines and found to be within the limit.

**Method validation**

In acquiescence with ICH recommendations, the validity parameters were established.

**RESULTS AND DISCUSSION**

**System suitability**

In six replicates, system suitability was achieved by injecting a regular solution containing 10 μg/ml Lercanidipine and 50 μg/ml of Atenolol. Ten tablets of each drug were accurately weighed and triturated to get a fine powder. A 10 mg Lercanidipine HCl and 50 mg Atenolol equivalent weight tablet powder was transferred into a 100 ml volumetric flask and dissolved in the diluent. The solution was ultra-sonicated for 10 min and made the volume with diluent. The tablet sample solution was then filtered through 0.45 micron syringe filter and utilized for preparing sample solution for the assay.

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| System suitability parameter | Acceptance criteria | Lercanidipine HCl | Atenolol |
|-----------------------------|---------------------|-------------------|----------|
|                             | Mean                | Std Dev           | % RSD    | Mean                | Std Dev           | % RSD    |
| USP Plate count             | NLT 2000            | 2475              | 37.113   | 1.50                | 7894              | 59.126   | 0.75    |
| USP Tailing                 | NMT 2.0             | 1.49              | 0.016    | 1.08                | 1.22              | 0.008    | 0.62    |
| USP Resolution              | NLT 2.0             | -                 | -        | 13.29               | 0.038             | 0.29     |
| Retention time              | NLT 2.0             | 2.916             | 0.008    | 0.28                | 6.476             | 0.008    | 0.12    |

mean±SD (n=6)

![Fig. 2: Chromatogram of system suitability](chart.png)
Specificity

At the retention time of Lercanidipine HCl and atenolol, no intervention [38] from the blank occurred. The process is also unique.

Linearity

Linearity was determined by plotting a curve between peak areas to its respective concentration. From this calibration curve [39, 40], it was noticed that the curve was linear over the 1-15 µg/ml Lercanidipine and 5-75 µg/ml atenolol concentration range. The calibration curve regression equations were $Y = 131823.93x + 25289.92$ ($R^2 = 0.999$) for Lercanidipine and $Y = 78721.38x + 26974.78$ ($R^2 = 0.999$) for atenolol.

Precision

The precision of this approach was evaluated in terms of inter and intraday variations. The intraday studies were calculated by six repeated tests of the Lercanidipine and atenolol sample solution under the same experimental conditions on the same day. In the same Laboratory, the intermediate precision of this approach was carried out by examining the analysis with various analyst and different instruments [41-43]. As the percent RSD values were found to be <2 percent, the method is highly accurate. At each added concentration, good recovery of the drug was achieved; suggesting that the procedure was successful. Below table represents the outcomes given.

Table 2: Linearity data

| Linearity level | Lercanidipine HCl | Atenolol |
|-----------------|-------------------|----------|
| Conc. (µg/ml)   | Peak area counts  | Conc. (µg/ml) | Peak area counts |
| Linearity-1     | 1                 | 169092   | 5            | 463266         |
| Linearity-2     | 2.5               | 380900   | 12.5         | 1015947        |
| Linearity-3     | 5                 | 678518   | 25           | 2004579        |
| Linearity-4     | 10                | 1338064  | 50           | 4026983        |
| Linearity-5     | 12.5              | 1661996  | 62.5         | 5014944        |
| Linearity-6     | 15                | 2012360  | 75           | 5829021        |
| Slope           | 131823.93         |          |              |                |
| Intercept       | 25289.92          |          |              |                |
| CC              | 0.9998            |          |              |                |

Table 3: Results of precision

| Parameter       | Lercanidipine HCl | Atenolol |
|-----------------|-------------------|----------|
|                 | Mean % recovery   | Std dev  | Conc. (µg/ml) | Mean % recovery | Std dev | Conc. (µg/ml) |
| Method precision| 100.2             | 0.135    | 10            | 99.8            | 0.105   | 50             |
| Intermediate precision | 99.6          | 0.009    | 10            | 100.8           | 0.134   | 50             |

n=6
Accuracy
By measuring the recovery experiments at three stages (50 percent, 100 percent, and 150 percent), the precision of the process was carried out. APIs were prepared at concentrations of 5, 10, 15 µg/ml of Lercanidipine and 25, 50, 75 µg/ml of atenolol. For each spike level, the test solution was injected three times and as per the test process, the assay was performed and the RSD values were less than 2 percent. Recovery percentage, mean and relative standard deviation have been determined. Recovery values showed that the approach within the desired range was specific.

Robustness
By varying flow rate and mobile phase composition, the robustness of the chromatographic process was calculated. It was found that RSD was within the appropriate range.

Forced degradation
The forced degradation study [47-49] was carried out according to ICH guidelines, include acid, base, peroxide, reduction, thermal and hydrolysis degradation. From the chromatograms, it is evident that selected drugs were stable under the applied stress conditions though the degradation peaks [50-52] were obtained. The formed degradants were characterized by using LC-MS/MS.

Acid degradation
Acid degradation of Lercanidipine and Atenolol were studied in 1N HCl. 12.6% of Lercanidipine and 14.7% of Atenolol degradation was observed in HPLC. Three degradation peaks were formed.

Alkali degradation
Alkali degradation of Lercanidipine and Atenolol were studied in 1N NaOH. 13.4% of Lercanidipine and 12.1% of Atenolol degradation was observed in HPLC. Three degradation peaks were formed.

Peroxide degradation
Peroxide degradation of Lercanidipine and Atenolol were studied in 30% hydrogen peroxide. 11.8% of Lercanidipine and 13.6% of Atenolol degradation was observed in HPLC. Two degradation peaks were formed.

Reduction degradation
Reduction degradation of Lercanidipine and Atenolol were studied in 30% sodium bisulphate solution. 14.5% of Lercanidipine and 12.3% of Atenolol degradation was observed in HPLC. Two degradation peaks were formed.

Thermal degradation
Sample was exposed to 105 °C for 6 h, 13.3% of Lercanidipine and 14.4% of Atenolol degradation was observed. No degradation products were formed in thermal degradation.

Hydrolysis degradation
Hydrolysis degradation of Lercanidipine and Atenolol was observed in HPLC grade water. 11.2% of Lercanidipine and 15.9% of Atenolol degradation was observed. No degradation peaks were observed in hydrolysis degradation.

Table 4: Results of accuracy

| Accuracy level | Lercanidipine HCl | Atenolol |
|---------------|------------------|----------|
|               | % Recovery       | Std Dev  | % Recovery | Std Dev |
| 50%           | 100.2            | 0.015    | 99.6       | 0.006   |
| 100%          | 99.8             | 0.112    | 100.4      | 0.137   |
| 150%          | 99.9             | 0.009    | 100.5      | 0.096   |

n=6

Table 5: Results of robustness

| Change in parameter | Lercanidipine (% RSD) | Atenolol (% RSD) |
|---------------------|-----------------------|------------------|
| Flow plus (1.2 ml/min) | 0.24                  | 0.49             |
| Flow minus (0.8 ml/min) | 0.19                  | 0.78             |
| Organic plus (55:45) | 0.83                  | 0.03             |
| Organic minus (45:55) | 1.03                  | 0.5              |

RSD-Relative standard deviation; All the values are presented as Mean, (n=3)

Table 6: Results of forced degradation

| Stress condition  | Lercanidipine (% degradation) | Atenolol (% degradation) |
|-------------------|-----------------------------|--------------------------|
|                   | Mean | Std dev | Mean | Std dev |
| Acid degradation  | 12.6 | 0.274   | 14.7 | 0.334   |
| Alkali degradation| 13.4 | 0.512   | 12.1 | 0.275   |
| Peroxide degradation| 11.8 | 0.187   | 13.6 | 0.548   |
| Reduction degradation| 14.5 | 0.356   | 12.3 | 0.228   |
| Thermal degradation| 13.3 | 0.509   | 14.4 | 0.376   |
| Hydrolysis degradation| 11.2 | 0.153   | 15.9 | 0.444   |

n=3

MS/MS degradation product
The fragmentation mechanism of degradation product 1of m/z-315 observed under acidic, alkali degradation conditions is shown in fig. 6. Abundant substance ions are seen on the spectrum at m/z-239 (C₆H₅LOSS), m/z-148 (C₆H₄Cl loss), m/z-78 (C₆H₅N loss). The proposed structures were confirmed by the accurate mass measurements and MS/MS studies.

MS/MS degradation product
Fig. 7 shows the fragmentation process of degradation product 2of m/z-406, which was observed under conditions of peroxide degradation. Abundant productions are seen on the spectrum at m/z-318 (C₆H₅O₃loss), m/z-230 (C₆H₅O₃loss), m/z-122 (C₆H₅NO₂loss), m/z-108 (C₆H₅NO₂loss, from m/z-230). The proposed structures were confirmed by the accurate mass measurements, MS/MS studies.
Fig. 5: Mass spectra of (A) D1 (B) D2 (C) D3 (D) D4 (E) D5 (F) D6 (G) D7

Collision induced dissociation of lercanidipine and atenolol

Scheme 1

Fig. 6: Mechanism for proposed fragmentation of DP₁ of m/z-315
Scheme 2

Fig. 7: Proposed fragmentation mechanism of DP₂ of m/z-406

Scheme 3

Fig. 8: Proposed fragmentation mechanism of DP₃ of m/z-556

MS/MS degradation product

Fig. 8 shows the fragmentation mechanism of degradation product 3 of m/z-556, which has observed under conditions of reduction degradation. Abundant product ions are shown in the spectrum at m/z-435 (C₆H₅NO₂ loss), m/z-358 (C₆H₅ loss), m/z-282 (C₆H₅ loss), m/z-210 (C₆H₅N loss), m/z-153 (C₄H₈ loss), m/z-94 (C₂H₃O₂ loss). The proposed structures were confirmed by the MS/MS experiments in combination with accurate mass measurements.

MS/MS degradation product

The fragmentation mechanism of degradation product 4 of m/z-243 observed under acidic, alkaline degradation conditions is shown in fig. 9. Abundant substance ions shown on the spectrum at m/z-186 (C₂H₄NO loss), m/z-76 (C₂H₄OCl loss), m/z-133 (C₂H₄Cl loss, from m/z-243). The proposed structures were confirmed by the MS/MS experiments in combination with accurate mass measurements.

Scheme 4

Fig. 9: Proposed fragmentation mechanism of DP₄ of m/z-243
Scheme 5

**MS/MS degradation product**

Fig. 10 shows the fragmentation process of degradation product 5 of m/z-225, that was observed under conditions of acid, alkali degradation. Abundant substance ions are seen on the spectrum at m/z-168 (C_2H_4NO loss), m/z-77 (C_3H_8O_3 loss), m/z-134 (C_3H_8O_3 loss, from m/z-225). The proposed structures were confirmed by the MS/MS experiments in combination with accurate mass measurements.

Scheme 6

**MS/MS degradation condition**

Fig. 11 shows the possible fragmentation mechanism of degradation product 6 of m/z-358, which was observed under conditions of peroxide degradation. Abundant substance ions on the spectrum at m/z-301 (C_2H_4NO loss), m/z-244 (C_2H_4NO loss), m/z-151 (C_3H_6O loss), m/z-95 (C_4H_8O loss). The proposed structures were confirmed by the MS/MS experiments in combination with accurate mass measurements.

Scheme 7

**Fig. 12: Proposed fragmentation mechanism for product degradation 7 of m/z-248**
MS/MS degradation product

Fig. 12 shows the mechanism of fragmentation of degradation product 7 of m/z-191 (loss of CH₃N), m/z-209 (loss of CH₂N), m/z-132 (loss of CH₃O). m/z-93 (loss of CH₃N), the proposed structures were confirmed by accurate mass measurements, MS/MS experiments.

CONCLUSION

In this study a fast novel, economical, sensitive and easily available method of HPLC has been produced for the simultaneous determination of Lercanidine and atenolol in bulk and a type of pharmaceutical dosage form. The advantage of this process was no HPLC methods were reported. This method consists of shorter run time, low price, accessibility, sensitivity, reliability and reproducibility. These properties are important when a large number of samples are to be analyzed. The validation of all the parameters like linearity, accuracy, specificity, robustness was done and found to be within the acceptance criteria. The RSD values were found to be less than 2.0 percent for all the parameters, which indicates the validity of the process and the results obtained by this process are seen to be in good agreement. So, the proposed method could be easily used for the routine analysis and pharmaceutical formulations of Lercanidine and atenolol in quality control laboratories without any preliminary separation.

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