RESEARCH ARTICLE

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Development and Validation of RP-HPLC Method for Simulataneous Estimation of Lercanidipine HCl and Atenolol in Pharmaceutical Formulations

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Abstracts: A simple, precise and RP-HPLC (reverse phase – high performance liquid chromatographic) method was developed and validated for the simultaneous determination of lercanidipine HCl (LDPH) and atenolol (AT) in pharmaceutical dosage form. The method involves the use of easily available inexpensive laboratory reagents. The separation was achieved on an Phenomenex Luna® C18 column with a particle size 5 µm, length 250 mm and internal diameter (i.d.) 4.6 mm with isocratic flow. The mobile phase at a flow rate of 1 mL/min consisted of 10 mM potassium dihydrogen phosphate (pH adjusted to 3.1 with 0.1 M ortho-phosphoric acid) and acetonitrile (65:35; v/v). A linear response was observed over the concentration range 2–18 µg/mL of LDPH and the concentration range 10–90 µg/mL of AT. Limit of detection and limit of quantitation for LDPH were 0.5 and 1.5 µg/mL, respectively and for AT were 1 and 3 µg/mL, respectively. The method was successfully validated in accordance to ICH guidelines acceptance criteria for system suitability, specificity, linearity, accuracy, precision and robustness. The analysis concluded that the method was selective for simultaneous estimation of LDPH and AT.

INTRODUCTION

Lercanidipine HCl (LDPH) is 3, 5-pyridinedicarboxylic acid, 1, 4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-2-[(3, 3-diphenylpropyl)methylamino]-1,1-dimethylethyl methyl ester hydrochloride (Figure 1), is a calcium channel blocker, utilized for the treatment of hypertension. [1, 2]

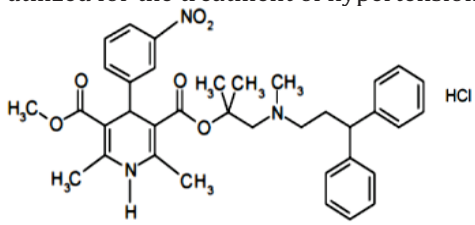

Figure 1: Structure of Lercanidipine HCl

Atenolol (AT) is 2-(4-{2-hydroxy-3-[(propan-2-yl) amino]propoxy} phenyl) acetamide (Figure 2), is a selective β_1 receptors blocker, utilized for the treatment of hypertension. [3, 4] Various publications are available regarding determination method of LDPH and AT but most of the methods are applicable for the analysis of LDPH or AT either alone or in combination with other drugs in pharmaceutical dosage form or in biological fluids. [5-8]

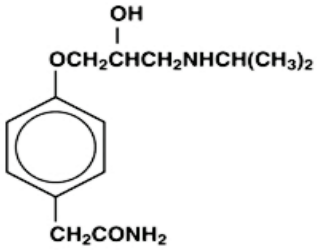

Figure 2: Structure of Atenolol

Only one method is reported for the simultaneous spectrometric estimation of LDPH and AT in tablet dosage form. [9] To our present knowledge, no RP-HPLC analytical method for the simultaneous determination of LDPH and AT in combine dosage forms has been reported and hence the present research work was endeavored for development and validation of RP-HPLC method for simultaneous estimation of LDPH and AT. In the presence work, an analytical method based on RP-HPLC was developed and validated for simultaneous determination of LDPH and AT in pharmaceutical formulation.

EXPERIMENTAL DETAILS

Chemicals and Reagents

LDPH and AT were gifted form Torrent Research Center, Gandhinagar, India. Acetonitrile (HPLC grade), methanol (HPLC grade) and potassium dihydrogen phosphate were purchased from Merck Chemicals, Mumbai, India. The marketed formulation of tablet, Lotensyl–AT® (Sun Pharmaceuticals, Mumbai, India) was purchased from local market. Double deionized water was utilized for entire study.

Instrumentation

A High Performance Liquid Chromatograph (HPLC) (Shimadzu Corporation, Tokyo, Japan) with work station of Shimadzu LC SOLUTION was employed for present investigation. The system consisted Shimadzu UFLC 20-AD as binary solvent delivery system, Shimadzu 7D Rheodyne Injector as loop injector and Photo Diode Array detector as a source of detection.

Chromatographic Conditions

The mobile phase consisting a mixture of 10 mM potassium dihydrogen phosphate (pH adjusted to 3.1 with 0.1 M ortho-phosphoric acid) and acetonitrile (65:35; v/v), was used throughout the analysis. Samples were separated using Phenomenex Luna® C18 column with a particle size 5 µm, length 250 mm and internal diameter (i.d.) 4.6 mm. The mobile phase was injected to the system using binary pumping mode at a flow rate of 1 mL/min. For all samples, run time and injection volume was kept constant as 12 min and 20 µL, respectively (Table 1).

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Table 1: Chromatographic Conditions for Analytical Method for Simultaneous Estimation of LDPH And AT

Mobile Phase	Phosphate buffer and acetonitrile (65:35, v/v)
Column	Phenomenex Luna® C18 Particle size - 5 µm, Length - 250 mm, i.d. - 4.6 mm
λ Scanning Range	200-800 nm
Flow Rate	1 mL/min
Injection Volume	20 µL

Table 2: Calibration Curves for Simultaneous Estimation of LDPH And AT

LDPH		AT	
Concentration (µg/mL)	**Peak Area**	**Concentration (µg/mL)**	**Peak Area**
2	19234.56 ± 1004.45	10	27542.64 ± 2345.65
4	39434.67 ± 3455.32	20	58367.13 ± 2355.67
6	67436.44 ± 4445.43	30	97325.41 ± 3467.76
8	88546.25 ± 5802.26	40	123436.34 ± 4456.14
10	121345.65 ± 4543.67	50	151446.76 ± 4246.88
12	147254.26 ± 3335.68	60	177547.15 ± 5135.78
14	181943.33 ± 4446.78	70	214657.25 ± 3236.77
16	208547.56 ± 6754.54	80	253456.65 ± 3467.87
18	228456.64 ± 4647.36	90	275662.87 ± 5888.32

Table 3: Precision of Method for Simultaneous Estimation of LDPH and AT

		Interday Precision		Intraday Precision	
Drug	**Nominal Concentration (µg/mL)**	**Mean Concentration (µg/mL)**	**% RSD (n=6)**	**Mean Concentration (µg/mL)**	**% RSD (n=6)**
	8	7.98	0.34	7.87	0.47
LDPH	10	10.23	0.23	10.52	0.73
	12	12.04	0.54	12.45	0.84
	40	7.98	0.34	7.87	0.47
AT	50	10.23	0.23	10.52	0.73
	60	12.04	0.54	12.45	0.84

Table 4: Accuracy of Method for Simultaneous Estimation of LDPH and AT

Drug	Nominal Concentration (µg/mL)	Level of Addition (%)	% Recovery (n=3)	% RSD
	10	80	99.55	0.34
LDPH	10	100	101.35	0.45
	10	120	100.40	0.67
	50	80	99.57	0.39
AT	50	100	99.78	0.36
	50	120	100.03	0.43

Preparation of Mobile Phase

For preparing a mobile phase, HPLC grade potassium dihydrogen phosphate (adjusted to pH 3.1 with 0.1 M ortho-phosphoric acid) and acetonitrile (65:35; v/v), was filtered through a 0.2 µm membrane filter and subjected to degassing in an ultrasonic bath (Frontline FS-4, Mumbai, India) for a period of 15 min. [10-13]

Preparation of Standard Solutions

A primary stock solution containing LDPH (100 µg/mL) and AT (500 µg/mL) was prepared by transferring 10 mg LDPH and 50 mg AT as standard into a 100 mL volumetric flask. The volume was made upto the mark with HPLC grade methanol and the system was ultra-sonicated for a period of 10 min on ultrasonicator bath. The stock solution was suitably diluted with HPLC grade methanol to obtain working range of standard solutions as solution containing LDPH (10 µg/mL) and AT (50 µg/mL) by pipetting 10 mL stock solution into a 50 mL volumetric flask and diluted up to the mark with mobile phase solution. [10-13]

Sample Preparation

Twenty tablets (Lotensyl-AT®) were weighed and crushed to a fine powder. Tablet powder equivalent to 10 mg of LDPH and 50 mg of AT was weighed and transferred to a 100 mL and volumetric flask. The volume was made upto the mark with HPLC grade methanol and the content was mixed and sonicated for a period of 15 min on ultrasonicator bath. The test solution was filtered through 0.45 µm membrane filters and filtered solution was transferred to a 50 mL volumetric flask and diluted to volume with mobile phase solution to give final test solution containing 10 µg/mL LDPH and 50 µg/mL AT. [10-13]

Table 5. Robustness of Method for Simultaneous Estimation of LDPH And AT

Type of Variations	Level of Variations	LDPH		AT	
		% Assay	Rt [a]	% Assay	Rt
Flow Rate (mL/min)	0.8	99.67	8.3	98.57	4.5
	1.0	100.23	8.2	100.56	4.4
	1.2	99.32	8.1	99.63	4.3
Mobile Phase (Buffer: ACN, v/v)	60:40	99.98	8.2	98.36	4.4
	65:35	100.03	8.2	100.34	4.4
	70:30	100.04	8.3	99.43	4.3
pH of Buffer	2.9	99.31	8.3	99.32	4.3
	3.1	99.97	8.2	99.78	4.4
	3.3	98.78	8.2	99.66	4.2

[a] Rt: Retention time (min)

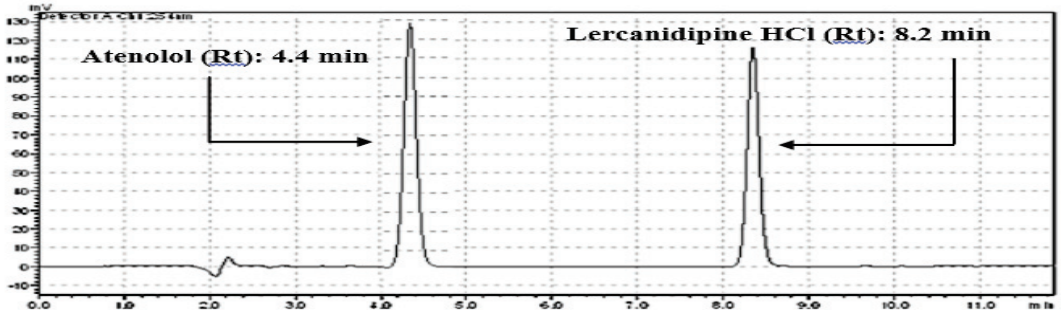

Figure 3: HPLC chromatogram of LDPH and AT

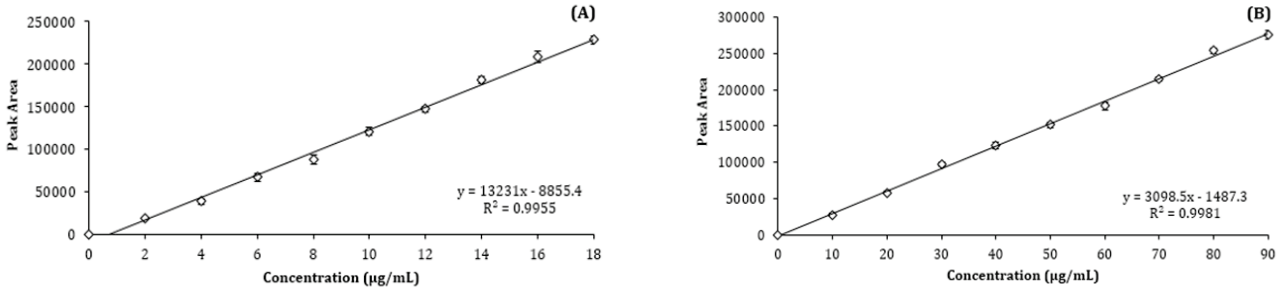

Figure 4: Calibration curve of (A) LDPH and (B) AT

Validation Parameters

System Suitability

The system suitability was evaluated by six replicate analyses of a LDPH/AT mixture at a concentration of 10 µg/mL of LDPH and 50 µg/mL of AT. [10-13]

Selectivity

The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. [10-13]

Linearity and Range

The calibration curves were constructed with nine concentrations (simultaneously prepared) ranging from 2-18 µg/mL and from 10-90 µg/mL for LDPH and AT, respectively. The values of peak areas were plotted against their respective concentrations in order to construct the calibration curve for each drug individually. Linear regression analysis was performed for each set of data using Microsoft Excel® version 2010 (Microsoft Corporation, Washington, USA). The study was repeated in triplicates to confirm reproducibility of results. The concentrations of the samples were calculated using linear regression analysis. [10-13]

Precision and Accuracy

Precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day). Three different concentrations of LDPH and AT were analyzed in six independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision). Every sample was injected in triplicate. The accuracy of the method, which is defined as the nearness of the true value and found value, was evaluated by recovery study which was performed by standard addition method. Known amount of drug was added to previously analyzed samples and its recovery was compared. [10-13]

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.2 mL/min), mobile phase composition (acetonitrile ±5%) and buffer pH (altered by ±0.2). These chromatographic

variations were evaluated for resolution between drug peaks with respect to % assay and retention time. [10-13]

Limits of Detection (LOD) and Quantitation (LOQ)

The detection and quantification limits were evaluated from calibration curves plotted in concentration ranges of 2-18 µg/mL LDPH and 10–90 µg/mL AT. The acceptance criterion for these replicate injections was RSD not more than 30% for LOD concentration and not more than 10% for LOQ concentration. The formulae used were LOD= 3.3 δ/m and LOQ= 10 δ/m (where δ = standard deviation of response and m = slope of calibration curve). [10-13]

RESULTS AND DISCUSSIONS

Analysis is an important component in formulation and development of any drug molecule. A suitable and validated method has to be available for the analysis of drug(s) in bulk, drug delivery systems, *in vitro* and *in vivo*. If such suitable method for specific need is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples. [10-13]

Chromatographic Conditions

The analytical conditions were selected, keeping in mind the different chemical nature of LDPH and AT. The column selection has been done on the basis of backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between LDPH and AT peak. After evaluating all these factors, Phenomenex Luna® C18 column was found to be optimum. In addition to this, the acidic pH range was found suitable for solubility, resolution, stability, theoretical plates and peak shape of both components. Initial trials were taken using methanol and potassium dihydrogen phosphate buffer (pH 3.1) as a mobile phase but we didn't get good peak shapes. When methanol was replaced by acetonitrile there was a marked improvement in the peak shapes of both drugs. This might be attributed to property of acetonitrile to reduce the longer retention time and to attain good peak shape. Finally, the mobile phase composition consisted of a mixture of 10 mM potassium dihydrogen phosphate buffer (pH 3.1) and acetonitrile (65:35, v/v) was selected for the study (Table 1). The retention time (Rt) for LDPH and AT were found to be 8.2 min and 4.4 min, respectively (Figure 3).

Validation

System Suitability

For both selected drugs the system was found to be highly suitable as the percent relative deviation (%RSD) was less than 2% for the peak area and the retention time for both LDPH and AT. [10-13]

Selectivity

The peak purity of LDPH and AT was found satisfactory and there was no interference from excipients of pharmaceutical dosage with any drug peaks. This revealed that the developed method was selective for each of drug. [10-13]

Linearity and Range

The mean regression equation of three standard curves for LDPH was y = 13231x – 8855.4 and for AT was y = 3098.5x – 1487.3. Where y presented the peak area of drug and x was the concentration of drug. The precisions (% CV) of the slope and intercept were less than 2% for both drugs which indicated minimum variations. [10-13] Both of the calibration curves were linear over the studied concentration range (2-18 µg/mL for LPDH and 10-90 µg/mL for AT) with a mean correlation coefficient more than 0.99 (Table 2 and Figure 4).

Precision and Accuracy

The % RSD results of intraday and interday precision for both the drugs were within 2.0%, confirming good precision of the developed analytical method (Table 3). Similarly high values of % recovery and low values of % RSD indicated accuracy of the developed method (Table 4).

Robustness

Robustness study, conducted by deliberate changes in flow rate, mobile phase composition and pH of mobile phase revealed that there was no significant variation in % assay and retention time (Rt) (Table 5). [10-13]

Limits of Detection (LOD) and Quantitation (LOQ)

The LOD and LOQ of LDPH using calibration curve in the range of 2–18 µg/mL were 0.5 and 1.5 µg/mL, respectively, while those of AT using calibration curve in the range of 10–90 µg/mL were 1 and 3 µg/mL, respectively. RSD (%) of six replicate injections of LDPH at LOD (0.5 µg/mL) and LOQ (1.5 µg/mL) were 1.28 and 3.79, respectively. Similarly % RSD of six replicate injections of AT at LOD (1 µg/mL) and LOQ (3 µg/mL) were 1.93 and 2.53, respectively. These values indicated that the method was very sensitive to quantify both the drugs. [10-13]

CONCLUSIONS

Hence, the proposed method for the simultaneous determination of LDPH and AT, in the presence of each other and, also in pharmaceutical dosage forms, give accurate and precise results. This RP-HPLC method gives a good resolution between LDPH and AT within a short analysis time. No interferences from excipients were observed in any of tablet samples with bypass the need for additional sample preparation, such as the extraction of the active constituents. Therefore, the proposed HPLC method can be used for routine simultaneous analysis of these drugs, and can be used as an alternative tool for the drug quality control laboratories.

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