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ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.2, pp 766-771, April-June 2011

Development and Validation of a RP-HPLC method for the simultaneous estimation of Atenolol and Lercanidipine hydrochloride in Pharmaceutical dosage forms

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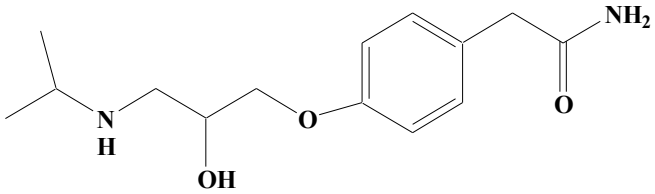
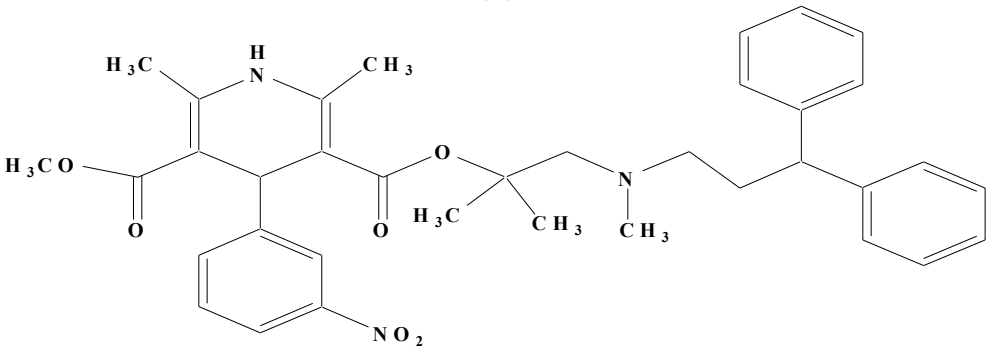
Abstract : A simple, precise, reliable, rapid and reproducible reversed-phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of Atenolol (ATL) and Lercanidipine Hydrochloride (LER) present in tablet dosage forms. Chromatographic separation achieved isocratically on Luna C_{18} column (5 µm, 150mm x 4.60mm) and ACN/phosphate buffer (60:40, v/v, pH 3.6) as mobile phase, at a flow rate of 0.5ml/min. Detection was carried out at 235 nm. Linearity for ATL and LER were in the range of 50-250 µg/ml and 10-50 µg/ml respectively. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The retention times for ATL and LER was found to be 2.27 and 5.97 min respectively. The mean recoveries obtained for ATL and LER were 99.85±0.16 and 99.47±0.32% respectively and RSD was less than 2. The correlation coefficients for all components are close to 1. The relative standard deviations for three replicate measurements in three concentrations of samples in tablets are always less than 2%. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of ATL and LER in tablets.
Key Words: Atenolol, Lercanidipine Hydrochloride, RP-HPLC, Simultaneous Estimation.

Introduction

Atenolol (ATL), (RS)-4-(2hydroxy-3-isopropylamino pro-poxy) phenyl acetamide (Figure 1A), is a cardioselective β-blocker. It is reported to lack intrinsic sympathomimetic activity and membrane-stabilizing properties. This drug is used to treat numerous cardiovascular disorders, for example hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction. It is official in USP, IP and BP[1-3]. Lercanidipine is chemically 2-{(3, 3-diphenylpropyl) methylamine}-1, 1-dimethylethyl, methyl-1, 4-dihydro-2, 6-dimethyl-4- (3-nitrophenyl)-3, 5- pyridine dicarboxylic ester (Figure 1B) with molecular formula $C_{36}H_{41}N_3O_6$. It is a new third generation 1, 4-dihydropyridine calcium channel antagonist used as antihypertensive agent. It is official in Merck Index and Martindale[4-6]. Atenolol alone or in combination with other drugs is reported to be estimated by HPLC in pharmaceutical dosage form[7-17], plasma [18-20], Serum[21], urine [22], UV spectrophotometry[23-32], spectrofluorimetry[33], gas–liquid chromatography[34], Chemometric[35], UPLC[36], HPTLC[37], capillary zone electrophoresis[38].



(A)



(B)

Figure -1 Chemical structures of (A) Atenolol (B) Lercanidipine

Some analytical methods for quantitative determination of Lercanidipine Hydrochloride in pharmaceutical formulations are described in literature like UV-Spectrophotometry[39-41], voltametric polarographic method[42-43], HPLC[44-48], in biological samples, plasm [49] and serum [50]. Two methods have been reported for simultaneous analysis of ATL and LER in its combination which includes TLC-densitometry and second order derivative spectrophotometry[51-52]. Extensive literature survey reveals that no RP-HPLC method is reported for simultaneous determination of ATL and LER in tablet dosage form. Fixed dose combination containing atenolol (50mg) and lercanidipine (10mg) is available in tablet form in the market. Therefore, an attempt was made to develop a new, rapid and sensitive RP-HPLC method for the simultaneous determination of ATL and LER in tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines, which is mandatory also[53-54].

Experimental

Instrumentation

Liquid chromatographic system from Shimadzu (LC-20AT) comprising of manual injector, double reciprocating plunger pump LC-20ATVp for constant flow and constant pressure delivery and Photodiode array detector SPD-M20A connected to software LC solution for controlling the instrumentation as well as processing the data generated was used.

Reagents and chemicals

Analytically pure sample of ATL and LER was kindly supplied by Glenmark Pharmaceuticals Ltd. (Nashik, India). Acetonitrile, Potassium dihydrogen phosphate, Disodium hydrogen phosphate was of HPLC grade supplied by Merck Ltd., India. The pharmaceutical dosage form used in this study was a Lotensyl AT (Sun Pharmaceuticals Industries Ltd. Mumbai) tablets containing 50 mg atenolol and 10 mg lercanidipine hydrochloride were obtained from the local drug market. Triple distilled water was generated in house.

Chromatographic condition

The isocratic mobile phase consisted of ACN/phosphate buffer (pH 3.6) in the ratio of (60:40 v/v), flowing through the column at a constant flow rate of 0.5 ml/ min. A Luna C_{18} column (5 µm, 150mm x 4.60mm) was used as the stationary phase. Although the ATL and LER have different λmax viz 224, 275 and 238, 358 nm respectively, but considering the chromatographic parameter, sensitivity and selectivity of method for two drugs, 235 nm was selected as the detection wavelength for UV-PDA detector.

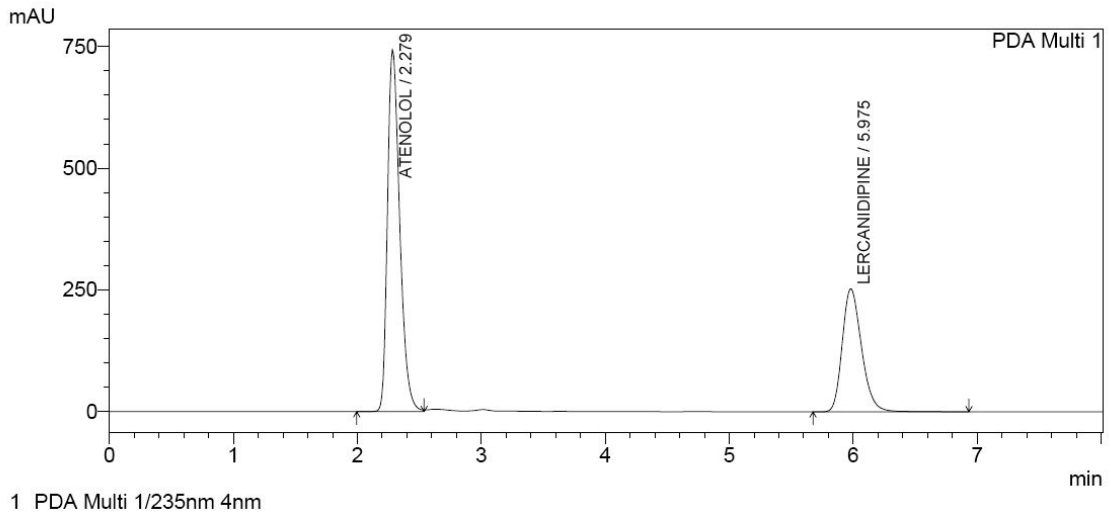


Figure 2. Representative chromatogram of Atenolol and Lercanidipine.

Standard preparation

Standard stock solution

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 100 ml of diluent which was a mixture of acetonitrile and phosphate buffer in the ratio of 60:40 (pH 3.6) to get concentration of 1000 µg/ ml.

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 50-250, 10-50 µg/ml for ATL and LER respectively.

Sample preparation

Twenty tablets (Lotensyl AT) were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 10 mg of LER and 50 mg ATL were transferred to 100 ml of volumetric flask. Drug was extracted with three 20ml quantities of mixture of diluent. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to mark and filtered through whatmann filter paper No. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.

Results and Discussion

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile/phosphate buffer (60:40, v/v, pH 3.6) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5/min were studied. A flow rate of 0.5 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed-phase C18 column, the retention times for Atenolol and Lercanidipine Hydrochloride were observed to be 2.27 and 5.97 min respectively. Total time of analysis was less than 6 min. The maximum absorption of Atenolol and Lercanidipine Hydrochloride together as detected at 235 nm and this wavelength was chosen for the analysis (Figure 2).

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table-1. The number of theoretical plates for ATL and LER were 2266 and 7023 respectively.

Linearity

ATL and LER showed a linearity of response between 50-250 and 10-50µg/ml respectively. The linearity was represented by a linear regression equation as follows.

Y (ATL)= 329898conc. + 347264 (r^2=0.9997)

Y (LER)= 90997 conc. - 6978 (r^2=0.9999)

Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method Table-2**.** The mean percentage recoveries obtained for Atenolol and Lercanidipine Hydrochloride were 99.85±0.16 and 99.47±0.32%, respectively.

Table-1 System suitability parameters

Parameter	Atenolol	Lercanidipine HCl
Retention time*	2.27±0.01	5.97±0.04
No. of theoretical plate*	2266.33±22.24	7023.33±54.00
Tailing factor*	1.39±0.01	1.26±0.01
HETP*	0.11±0.00	0.04±0.00
Linearity range	50-250 µg/ml	10-50-µg/ml

* Each value is the Mean ± S.D of six determinations

Table-2 Result of recovery studies with statically evaluation

Serial. No.	Conc. of drug in preanalyzed samples (µg/ml)		Std. drug sol. Added (µg/ml)		Recovered amount* (µg/ml)		% Recovered	
	ATL	LER	ATL	LER	ATL	LER	ATL	LER
1	50	10	50	10	49.97	9.89	99.927	99.100
2	100	20	100	20	99.92	19.82	99.970	99.683
3	150	30	150	30	148.93	29.88	99.673	99.633
						Mean	99.857	99.472
						%R.S.D	0.160	0.325

* Mean of Nine determinations (3 replicates at 3 concentration level)

Repeatability

Five dilutions in three replicates were analyzed in same day for repeatability and results were found within acceptable limits (RSD ‹ 2) as shown in Table-3.

Intermediate Precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits (RSD ‹ 2) as shown in Table-3.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method's capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of acetonitrile: phosphate buffer (pH 3.6) (60:40v/v), acetonitrile: phosphate buffer (pH 3.6) (55:45 v/v), was used as a mobile phase. Results of analysis were summarized in Table- 4.

Tablet Analysis

Content of ATL and LER found in the tablets by the proposed method are shown in Table-5. The low values of R.S.D. indicate that the method is precise and accurate.

Table-3 Result of precision

Validation Parameter	Percentage Mean ± S.D*. (n=15)		Percentage RSD*	
	ATL	LER	ATL	LER
Repeatability	99.839±0.321	99.66±0.084	0.200	0.310
Intermediate precision				
Day to Day	99.91±0.163	99.90±0.095	0.113	0.404
Analyst to Analyst	99.83±0.175	99.43±0.088	0.141	0.381

* Mean of Nine determinations (3 replicates at 3 concentration level)

Table-4 Results of robustness

Serial. No.	Validation Parameter	% Mean*		S.D.		% R.S.D.	
		ATL	LER	ATL	LER	ATL	LER
1	Robustness	99.75	99.41	0.234	0.119	0.191	0.573

* Mean of six determinations

Tablet-5 Result of marketed tablet analysis

Parameter	Lotensyl AT	
	ATL	LER
Mean % estimated	99.85	99.05
Standard deviation(S.D.)	0.11	0.56
% Coefficient of variation	0.11	0.57
*Standard error (SEσ)	0.03	0.13

*** Mean of fifteen determinations (3 replicates at 5 concentration level)**

Conclusion

RP-HPLC method was developed and validated for simultaneous estimation of atenolol and lercanidipine hydrochloride in tablet dosage form. The developed method is suitable for the identification and quantification of binary combination of atenolol and lercanidipine hydrochloride. A high percentage of recovery shows that the method can be successfully used on a routine basis. Proposed method is simple, fast, accurate, precise and sensitive and could be applied for quality and stability monitoring of atenolol and lercanidipine hydrochloride combination.

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