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[Research article]

Analytical Method Development and Validation for Simultaneous Estimation of Lercandipine and Atenolol Tablet Dosage Form by RP-HPLC

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ABSTRACT

A rapid high performance liquid chromatographic method has been development and validation for the estimation of Lercanidipine and Atenolol stimultaneously in combined dosage form. A kromosil C-8 column having dimensions of 4.6µmx250mm and particle size of 5µm in isocratic mode , with mobile phase containing a mixture of Di- Potassium Hydrogen Phosphate and Acetonitrile in ratio of (70:30v/v) (pH adjusted to pH 6.5±0.5 using phosphoric acid was used . Detection was done at 215 nm using PDA detector. The mobile phase was pumped at a flow rate of 1.0µl/min and injection volume is10µl. The selected chromatographic conditions were founds to effectively separate Lercandipine (Rt 3.8min)[2] and Atenolol (Rt: 6.1 min) having resolution of 7.8. The method was validated in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantitation.

Key Words: Lercanidipine, Atenolol, RP HPLC, Method development, Validation

INTRODUCTION

Atenolol is a (RS)-4-(2hydroxy-3-isopropylamino pro-poxy) phenyl acetamide is a cardio selective β1-blocker. It is a selective β-adrenergic receptor blocking agent without membrane stabilizing of intrinsic sympathomimetic (partial agonist) activities. This preference effect is not absolute however, and at higher doses. Atenolol inhibits β2-adreno receptors, chiefly located in the bronchial and vascular musculature [1].

Lercandipine is chemically 2-{(3,3-diphenylpropyl methyl amine}-1,1-dimethylethyl , methyl-1,4-dihydro-2,6-dimethyl-4- (3-nitro phenyl)-3,5-pyridine decarboxylic ester.[5] It is a new third generation calcium channel antagonist used as anti-hypertensive agent. Atenolol alone and combination with other drugs is reported to be estimated by HPLC[5] in pharmaceutical dosage form. The Uv-spectrophotometry[9], gas-liquid chromatography, HPTLC. capillary zone electrophoresis, some analytical methods for quantitative estimation of lercandipine in pharmaceutical formulation and human plasma [3]. A rapid and sensitivity RP-HPLC method for the stimultaneous determination of lercandipine and atenolol in tablet dosage form. The method was validated as per ICH guideline which is mandatory [1,4].

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MATERIAL AND METHODS

Instrument

Liquid chromatography consists of the following components: Waters Allinance 2695, and rheodyn injector. Chromatographic analysis was performed on a KromosilC8 (250mm x4.6µm) i.d and 5µm particle size the mobile containing mixture of Di-Potassium hydrogen phosphate and Acetonitrile in the ratio of 30:70. pH was adjusted with phosphoric acid to 6.5±0.5. it was filtered with Whatman filter no1 and degassed for10mins. The flow rate of the mobile phase was maintained at 1.0µl/min. Detection was carried out at 215nm in PDA detector.

Chemicals and Regents.

HPLC water (HPLC grade) – Merck Speciality Pvt., Mumbai.Acetonitrile (HPLC grade) - Merck Speciality Pvt., Mumbai.Di-potassium hydrogen phosphate (Hipur Fine Chemical Industry) Phosphoric acid (HPLC grade) – Merck Speciality Pvt., Mumbai.% purity of Lerandipine 99.5%. %purity of atenolol is 99.63%. Sample-commercial tablet of lotensyl AT (SunPharma)

Preparation of standard stock solution

A 10 mg of lercanidipine working standard solution was taken in a 10ml volumetric flask and dissolved it properly and diluted up to volume with the diluent, so as to give a concentration of 1000 mcg/ml of lercanidipine and 100 mcg/ml of atenolol. 10µl of this solution was injected and chromatogram was recorded. The retention time of atenolol was found to be 6.112 and lercanidipine was found to be 3.887 mins.

Preparation of Sample solution

Accurately weighed quantity of powder equivalent to 10mg of lercanidipine and 50 mg of atenolol from 20 tablets, was dissolved in10ml volumetric flask using diluent by sonication and made up to volume with diluent. The solution was filtered through the nylon milli-pore filter. Take 10µl of solution was injected in HPLC according to the chromatographic conditions and chromatograms were recorded. The amount of lercanidipine and atenolol present in each tablet formulation were calculated by comparing the peak areas of the standards and reports shown in table.

Amount of drugs in each tablet were found to be:

$$\frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Standard weight}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight}} \times \frac{\text{Purity}}{100} \times \text{Average weight}$$

Each value is the mean of six readings. Acceptance criteria: 90-110 % w/v.

Sample	Label-claim (mg/tab)	Peak Area	Amount present	% label claim w/v
lercanidipine	10mg	84042	10.04 mg	100.81 %w/v
atenolol	50mg	3438447	50.37mg	97.28 %w/v

RESULTS AND DISCUSSION

METHOD VALIDATION[4,5]

The method was validated with reference to ICH guidelines i.e. Linearity, Accuracy, precision, specificity, ruggedness respectively.

Specificity

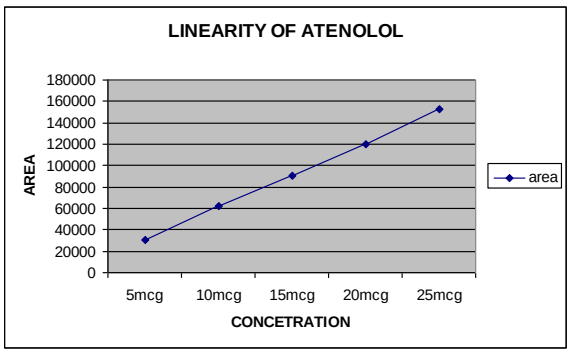
The specificity of the method was evaluated by analyzing the sample solution spiked with the blank solution at appropriate levels. The assay result was unaffected by the presence of extraneous materials. It was determined by 10µl of blank solution was injected and t chromatogram were recorded. Take 10mg of atenolol working standard was taken into 10 ml volumetric flask and added 1ml of lercanidipine standard solution using diluent by sonication and made up to volume with diluent.

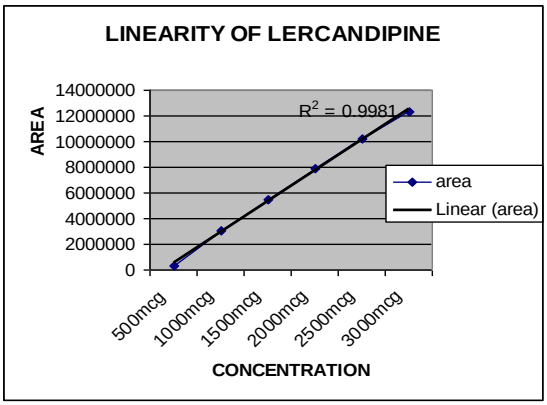
S.No.	Sample	Area obtained	Area %
1.	Blank	0	0
2.	lercanidipine	3675994	97.390
3.	Atenolol	98524	2.610
	Total	3774518	100%

LINEARITY AND RANGE

The linearity of an analytical method is its ability to Elicit test results that is directly proportional to the concentration of analyte in sample with in a given range. The linearity of an analytical method is determined by mathematical treatment of test result obtained by analysis of samples with analyte concentration across claimed range of peak area Vs concentration was plotted and percentage curve was calculated. Appropriate aliquots of standards stock solution of Lercandipine and Atenolol. The linear range of Lercandipine and Atenolol was found to be (500mcg-3000mcg), r^2-0.9981 and (5mcg -30mcg), r^2-0.9996.

Acceptance criteria: Coefficient of co-relation (r^2) not be less than 99





PRECISION

Precision of the method was validated by performing replicated assays of the homogeneous sample. Results were calculated in terms of %RSD of the content of Lercandipine and Atenolol

A. SYSTEM PRECISION

Preparation of standard solution

A 10 mg of lercanidipine working standard was accurately weighed and transferred in to 10 ml volumetric flask and 1ml atenolol working standard solution was added, dissolve using diluent by sonication and made up to volume with diluent. The solution was filtered through the nylon milli-

pore filter. To obtained final concentration of 1000 mcg/ml of lercanidipin and 100 mcg/ml of atenolol. About 10 μl of the solutions was injected and chromatograms were recorded.

The standard deviation and relative standard deviation were calculated from statistical formula

$$S.D\ (\sigma) = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

Where x = Sample

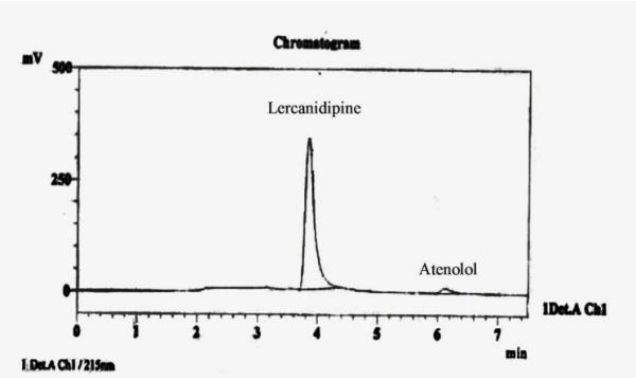
$\bar{x}$ = Mean value of samples

n = No. of Samples

$$R.S.D\ (\%) = \frac{\sigma}{\bar{x}} \times 100$$

B: Method Precision

The method precision was validated for intermediate precision by comparing the performance of method. Six replicate assay of homogeneous sample were performed using the same procedure and chromatographic conditions. Six successive injections of 10 µl of working sample solution were injected and chromatograms were recorded.



SYSTEM PRECISION OF ATENOLOL AND LERCANIDIPINE

S.No	PEAK AREAS OF	
	ATENOLOL	LERCANIDIPINE
1	99666	3692555
2	98636	3672587
3	98854	3685378
4	99794	3660673
5	98545	3704044
Mean	99099	3683047
S.D	588.6	16931.6
% RSD	0.59	0.46

Relative Standard Deviation	ATENOLOL	LERCANIDIPINE	Acceptance Criteria
	0.59	0.46	2.0%

METHOD PRECISION OF LERCANDIPINE AND ATENOLOL.

S. No	Weight in mg	Area obtained		Assay value in mg		% label claim W/V.	
		ATEN	LERCANI	ATEN	LERCAN	ATEN	LERCAN
1	694.09	84043	3438449	509.07	58.31	101.83	97.29
2	693.08	83749	3408346	508.04	57.29	100.75	96.59
3	695.16	85149	3537446	510.13	59.64	102.65	99.13
4	694.34	84534	3486501	509.53	58.71	101.96	97.64
5	695.06	83649	3416335	508.65	57.49	100.89	96.92
			Mean			101.6	97.5`
			S.D			4.650	4.025
			RSD			0.67	0.58

RSD	ATENOLOL	LERCANIDIPINE	Acceptance criteria
	0.67	0.58	NMT : 2.0%

ACCURACY

Accuracy was performed by the method of standard addition at three different levels, multiple level recovery studies .Accuracy of an analytical method was the closeness of test result obtained by developed method to the true value. Pre analyzed sample was spiked with Lercandipine and Atenolol in the same proportions as present in tablet dosage form. Spiked samples were prepared in triplicate at three intervals a range of 80- 120 percentage of the target concentration and injected in the HPLC system.

Acceptance criteria: Percentage recovery should be within 90-110% w/w.

Preparation of working standard stock solution

About10 mg of lercanidipine working standard was weighed and transferred in 10ml volumetric flask and added 1ml of atenolol standard solution, dissolved and diluted to volume with the diluent. Preparation of working mixture solution Aliquot volume of 8ml, 10ml, 12ml, standard stock solution was transferred in to 3 different 25 volumetric flasks. And made up to volume with diluent. That

gives 80%, 100%, 120% of working mixture solution. 10 µl of each solution was injected and chromatograms were recorded. The accuracy data of atenolol and lercanidipine was shown in tables.

RECOVER DATA OF ATENOLOL

S.No	Recovery	Area obtained	Avg. area	Amt required in mg	Percentage (%) recovery w/v
		72593			
1	80%	73487	73918	40.21	100.05
		75674			
		88563			
2	100%	87593	87549	45.57	99.9
		86493			
3		96542			
	120%	97685	96573	50.72	99.7
		95492			

RECOVRY DATA OF LERCANDIPINE

S.No	Recovery	Area obtained	Avg. area	Amt required in mg	Percentage (%) recovery w/v
		2865993			
1	80%	2759952	2675920	7.8	99.5
		2675317			
		3447786			
2	100%	3375867	3394115	8.7	99.6
		3358692			
		4269873			
3	120%	4196385	4252009	9.8	99.7
		4289769			

RUGGEDNESS

The ruggedness of an analytical method is degree of reproducibility of test result obtained by the analyst under a variety of normal test condition. Such as different laboratories different analysts different instruments, lots of reagents different elapsed assay times, different temperature, different days etc. The ruggedness of analytical method is determined by aliquots from homogenous lots of different analyst using operational and environmental conditions that may differ but are

also within the specified parameters of the assay. The degree of reproducibility of test results is then determined as function of assay variables. This reproducibility may be compared with the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method. The assay of lercanidipine and atenolol was performed in different ways by different analysts. It was determined by Working standard solution and working sample solution of lercanidipine and atenolol were prepared by different analyst on different days and 10 µl of working standard solution and working sample solution was injected and chromatograms were recorded ruggedness of method and report was shown in tables.

RUGGEDNESS OF METHOD (DIFFERENT ANALYSTS)

S.No	Instrument code	Analyst	Date of analysis	Recovery %	
				atenolol	lercanidipine
1	Waters 2695	Analyst – 1	11/06/13	99.8	99.7
2	Waters 2695	Analyst – 2	11/06/13	98.8	99.3
3	Waters 2695	Analyst – 1	12/06/13	99.9	99.6
4	Waters 2695	Analyst – 2	12/06/13	99.5	98.6

RUGGEDNESS OF METHOD (DIFFERENT INSTRUMENT)

S.No	Analyst	Instrument code	Date of analysis	Recovery %	
				Atenolol	Lercanidipine
1	Analyst - 1	Water 2695	11/06/13	99.7	99.8
2	Analyst – 1	Prominence	11/06/13	98.9	98.5

ROBUSTNESS

Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. For eg. Change in physical parameters like flow rate and wavelength. It is estimated by10 µl of various mixed working standard solution was injected and chromatogram was recorded and shown in the tables.

REPORT ON CHANGE IN FLOWRATE 0.8 ml/min.

Drugs	Avg area in 0.8 ml/min	Avg. area in 1ml/min	Std deviation	% RSD
lercanidipine	4156783	3476529	19278	0.49
Atenolol	91672	84973	586.4	0.63

REPORT ON SAMPLE CHANGE IN FLOWRATE 1.2 ml/min

Drugs	Avg area in 0.8 ml/min	Avg. area in 1ml/min	Std deviation	% RSD
lercanidipine	3171793	3476529	23721	0.15
Atenolol	81265	84973	764.5	1.7

REPORT ON CHANGE IN WAVE LENGTH OF 210 nm.

Drugs	Avg area in 0.8 ml/min	Avg. area in 1ml/min	Std deviation	% RSD
lercanidipine	3457928	3446798	10874	0.5
atenolol	83376	84568	493.7	0.3

REPORT ON CHANGE IN WAVE LENGTH 220 nm.

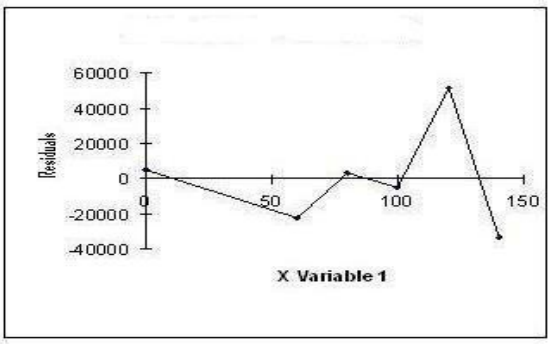
Drugs	Avg area in 0.8 ml/min	Avg. area in 1ml/min	Std deviation	% RSD
lercanidipine	2647689	3446798	1657	0.54
Atenolol	76657	84568	276.2	0.34

LIMIT OF DETECTION (LOD)

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated experimental condition. The detection limit is usually expressed as the concentration of analyte (e.g. parts per million). It is determined by based on the standard deviation of response and the slope. The detection limit was being expressed as LOD = $\frac{3.3\sigma}{S}$, Where

σ =the standard deviation of the response.

S= the slope of the calibration curve of the analyte.



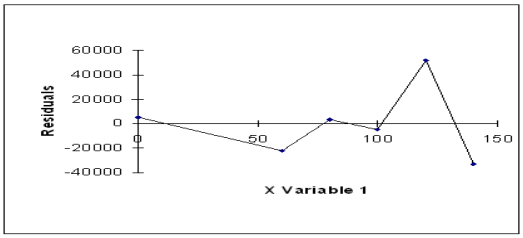
	atenolol	lercanidipine
LOD	9.42 mcg/ml	1.28 mcg/ml

LIMIT OF QUANTIFICATION

The quantification limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantiated with suitable precision and accuracy.

Based on the deviation of the response and the slope.

LOQ = $\frac{10\sigma}{S}$ Where σ =the standard deviation of the response. S= the slope of the calibration curve of the analyte.



	atenolol	**lercanidipine**
LOQ	28.55 mcg/ml	3.38 mcg/ml

SYSTEM SUITABILITY PARAMETER

For system suitability , five replicate of standard solutions of Lercandipine and Atenolol working standard was injected studied the suitability parameters like Plate Number (N), Resolution (R), Relative retention time (α) and Peak symmetry of sample (As) were studied with the help of standard chromatograms

System suitability factors	**Atenolol**	**Lercanidipine**
Area	109359	3806509
Retention time	6.1	3.8
USP Tailing factor	1.639	2.157
USP plate count	5772	3471
Resolution (R)	7.8	
Capacity factor (K)	0.59	

SUMMARY AND CONCLUSION

A RP-HPLC method was developed for the estimation of Lercanidipine and Atenolol in tablet dosage form using HPLC-Waters Alliance 2695 separations module, with Detector – Waters 2996 separations module and column Kromosil C_8, 25cm x 4.6mm, 5 µm.Injection volumn was 10µl and the mobile phase was Buffer solution (Di- Potassium hydrogen phosphate) and Acetonitrile in ratio (30:70) having a pH range 6.5±0.05. where as mobile phase was pumped at a flow rate of 1.0ml/mim maintaning column temparature at 60ºc±1ºc and detected at 215nm using PDA Dectector.

The peak Retention time of the Lercandipine is 3.8min and Atenolol is 6.1min. The excipientes in the formulation dose not interfer in the estimation of active drug. The determination of the Lercanidipine and Atenolol by RP-HPLCmethod analysis yielded well resloved peaks with in the short analysis time of<10min. The valve of standard deviation were satisfactorily low and recovery was close to 100%.the corelation co-efficient of linearity studies were found to be 0.998 for Lercandipine and 0.999 for Atenolol.

The developed method was validated for system suitability, linearity, precision, accuracy in accordance with international conference on harmonization guidelines(ICH).This method was suitable for the routine analysis of combination drugs(Lercandipine and Atenolol) in pharmaceutical formulations.

REFERENCES

[1] Sumeet Dwivedi, nirvedUpadhaya, AwadudayY. And pekamwar Sanjay . IJDDHR.ISSN:2231-6078, Settember(2011) pg:134-137.
[2] G.Mubean, Mamata Pal and M.N. Vimala. International Journal of Pharma and Bio Sciences Vol 1(1) 2010.
[3] Jaya Raj Kumar , Sokalingam Arumugam Dhanaraj Am .J. Pharmatech. ISSN: 2249-3387 , 2012 VOL 2(5).
[4] Panthaganda Sunitha, Kancherla Satyavathi, IRJP, vol 3(11) ISSN :2230-8407.
[5] Deepak Kumar Jain , Prathiba Petal , Abu Salam Khan. IJCHR , ISSN:0974-4290, VOL 3, April- June 2011.
[6] Abdul sallem , P.Permulal D.Boothapathy IJPTRIF ; ISSN:0974-4304, Vol 2(1), pp: 471-474, Jan-Mar2010.
[7] M.Radha Krishna and Ramesh Jagadesan, IJRPBS; vol.4(3) ISSN:2229-3701, Jul-Sep2013.
[8] A.K. Shah ; H.O Kaila, IJPS; May-June2010.
[9] T.Manikya Sastry and K.Rama Krishna ; IJCT , Vol 16, sep2009, pp:431-436.
[10] Juddy Joseph; M. Sundarapandian; IJPSR 2011 Vol.2(8), pp 2156-2161;ISSN:0975-8232. July2011.
[11] Amit Khandar; Maiteyi Zaveri; JPRHC; VOL 2(3) pp:248-252.
[12] R.S.Murthy, Mariam Ravi Kumar; IJSID ISSN: 2249-5347, Vol2(1) Jan-Feb2012.
[13] Vidya K. Bhusari; Sunil R. Dhaneshwar. URPJ 2011 vol1(3) ;pp:70-76.
[14] M.V. Kumudhavali ; K. Anand Babu , B.Jayakar, DerPharma Chemica 2011, vol3(4) , ISSN:0975-413X, pp: 63-68.
[15] Venkateswarlu.Ponneri, Thulasamma Paresu. EURJChem 2012,vol3(2) , pp:138-142.
[16] S. AppalaRaju , B. Karadi Inf. J.Chem Sci 2008 ,vol6(1), pp:441-446.
[17] S.Pujol,J.A, A.Aivarez- Lueje, Bio Electrical Laboratory , University of Chile
[18] A.Agrawal , K.Mitial, JPRHC 2010 VOL.2(3) ,PP:263-265.
[19] Nilesh Jain, Ruchi Jain, E.S.Analytical. Chem 2011, vol6(2) ,pp:84-90.
[20] Sherke.O.S, Sable.K.S, IRJP2012,vol3(4), ISSN:2230-8407.
[21] Vishnu. choudhari, Rashmi.H.Mahabal, IJPSR&R,ISSN:0976-044X, vol.3(1) , July-Aug 2010.
[22] Vascular Health and Risk Management 2005 vol1 (3), pp: 173-182.

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