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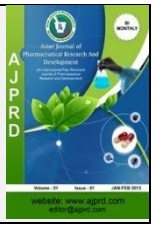

Open Access Research Article

Validated RP-HPLC Method for Simultaneous Estimation of Atenolol, Hydrochlorothiazide and Losartan Potassium in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, specific, accurate, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for the simultaneous estimation of Atenolol, Hydrochlorothiazide and Losartan potassium in bulk and pharmaceutical dosage form. The separation was carried out using Hypersil C18, 250 ×4.6 mm, 5µm column with mobile phase consisted mixture of Acetonitrile and Potassium dihydrogen ortho phosphate buffer in the ratio of 40:60 (V/V) delivered at a flow rate of 1.5 mL / min and effluents were monitored at 225 nm. The selected chromatographic conditions were effective separation of these drugs. And the retention times of Atenolol, Hydrochlorothiazide and Losartan potassium were found to be 1.46, 2.21 and 3.30 min respectively. The proposed method was found to be linear in the range of 50-150 µg/mL,12.5-37.50 µg/mL, and 50-150 µg/mL for Atenolol, Hydrochlorothiazide and Losartan potassium respectively. The recovery of drugs was found to be 97.56 %, 97.72 % and 98.06 % respectively. The proposed method was validated as per ICH guidelines and it was found to be accurate, precise and robust, and it was applied to the estimation of Atenolol, Hydrochlorothiazide and Losartan potassium in combined tablet dosage form and it can also be used for routine quality control analysis of these drugs in biological samples either alone or in combined pharmaceutical dosage forms.

Keywords: Atenolol, Hydrochlorothiazide and Losartan potassium, RP-HPLC, Validation and ICH guidelines.

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INTRODUCTION

Atenolol (ATEN),4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzeneacetamide, (Fig. 1) is an antihypertensive, antianginal, and antiarrhythmic [1]. It is a beta-adrenergic blocking agent, by blocking the stimulation of these nerves, atenolol reduces the heart rate and is useful in treating abnormally rapid heart rhythms, and also reduces the force of contraction of heart muscle and lowers blood pressure. By reducing the heart rate, the force of muscle contraction, and the blood pressure against which the heart must pump, atenolol reduces the work of heart muscle and the need of the muscle for oxygen. Since angina occurs when oxygen demand of the heart muscle exceeds the supply, atenolol is helpful in treating angina. Atenolol is official in the IP, BP, and USP [2-5].

Hydrochlorothiazide (HCTZ) is chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide1,1-dioxide (Fig.2). It is the potent orally diuretic and antihypertensive agent related to chlorothiazide. This inhibits active chloride reabsorption and thus increases the excretion of sodium chloride and water [6-7].

Losartan potassium (LOST) is monopotassium salt of 4-butyl-4-chloro-1- [[2'-(1H-tetrazole-5-yl) [1,1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-methanol (Fig.3). It is a selective, competitive angiotensin II receptor type 1 (AT1) receptor antagonist. Losartan administration results in a

decrease in total peripheral resistance and cardiac venous return [8-9].

A new combination dosage form of LOST, ATEN and HCTZ is indicated for the treatment and management of hypertension. Literature survey revealed that few analytical methods have been reported for estimation of ATEN, HCTZ and LOST individually or in combination with other drugs. The reported methods are Spectrophotometric [10-16], RP-HPLC [17-35], HPTLC [36-39]. There are few analytical methods have been reported as per our knowledge for simultaneous estimation of ATEN, HCTZ and LOST in combined pharmaceutical formulations, reported methods are HPLC [40-46], UPLC [47], HPTLC [48]. The present study was aimed to develop a simple, rapid, precise and accurate RP-HPLC method for simultaneous estimation of ATEN, HCTZ and LOST in bulk and tablet dosage form. The developed method was validated according to ICH guidelines [49-51].

MATERIAL AND METHODS

Chemicals and reagents: Working Standard samples of Atenolol, Hydrochlorothiazide and Losartan Potassium were obtained from Startech Labs India Pvt.Ltd, Hyderabad, India. The marketed formulation of REPALOL H tablets (Atenolol 50 mg, Hydrochlorothiazide 12.5 mg and 50 mg Losartan Potassium/tablet) were procured from local pharmacy Store. Analytical grade of Potassium dihydrogen orthophosphate, orthophosporic acid and HPLC grade of acetonitrile were procured from SD Fine Chemicals Ltd., Mumbai, India. HPLC grade water was obtained by triple distillation and purified additionally with Milli-Q water purification system.

Instrumentation: The analysis was performed by using a chromatographic system Water 2695 series HPLC comprised of vacuum degas, auto injector, and dual gradient pump with UV-Visible detector. The HPLC system was equipped with Empower 2 software.

Chromatographic conditions: ATEN, HCTZ and LOST was analysed with Hypersil C_{18} Column (250 x 4.6 mm internal diameter;5 µm particle size) for the chromatographic separation. The mobile phase was composed of a mixture of Acetonitrile and Potassium dihyrogen orthophosphate buffer in the ratio of 40:60 V/V and it was delivered at a flow rate of 1.5 mL/min and UV detection was performed at 225 nm. and mobile phase was used as diluent. Injection volume was 20 µL. The run time was 10 min. The retention times of ATEN, HCTZ and LOST were found to be 1.46, 2.21 and 3.30 min respectively.

Mobile phase preparation: Accurately weighed 1.36 g of Potassium dihydrogen orthophosphate and transferred into a 1000 mL clean and dry volumetric flask, added about 500 mL of HPLC grade water purified with Milli-Q purification system and Sonicated for degassing finally made up to the mark with water. And pH was adjusted to 5.6 with diluted orthophosporic acid solution. 400 mL of Acetonitrile and 600 mL of buffer were added in a 1000 mL flask.

Standard stock preparation: Accurately weighed and transferred 25 mg of ATEN, 6.25 mg of HCTZ and 25 mg of LOST working standards into 50 ml clean, dry volumetric flask, and added 10 ml of acetonitrile to dissolve and sonicated for 5 minutes and made up to the final volume with acetonitrile. From the above stock solutions, 1 ml was pipetted out in to a 10 ml volumetric flask and then made up to the final volume with diluent.

Sample Solution Preparation: Accurately 20 tablets were weighed individually and the average weight was calculated and powdered. The tablet powder equivalent to 50 mg of ATEN and 12.5 mg of HCTZ and 50 mg of LOST transferred into a 100 ml volumetric flask, to that 10 ml of acetonitrile was added and sonicated for 10 minutes at controlled temperature to dissolve the powder, further the volume made up with diluent, and filtered through 0.45 µ membrane filter. From this solution 1.0 ml was diluted to 10 ml with diluent.

Method Development and Optimization: The optimized HPLC conditions, several mobile phases of different composition, flow rates and ratios were tested to develop an optimization of chromatographic conditions such as tailing factor, good peak shape, and theoretical plates. Finally, mobile phase consisting a mixture of acetonitrile: potassium dihydrogen orthophosphate buffer (40:60 % V/V) at a flow rate of 1.5 mL/min was found to be satisfactory and proper system suitability parameter results were obtained.

METHOD VALIDATION

Developed method was validated as per ICH guidelines over the system suitability, linearity, accuracy, precision, limit of detection, limit of quantification, robustness, specificity [50,51].

System Suitability: System suitability is an integral part of the chromatographic system. It is verification of resolution, capacity factor, tailing factor, theoretical plate count, relative retentions etc are calculated and compared with standard specification of system.

Linearity: Linearity is the ability (within specified range) to obtain test results are directly proportional to the concentration of analyte in the sample. Linearity is evaluated by visual inspection of plot of signal as a function of analyte concentration. If there is a linear relationship test results are calculated by regression line by method of least squares.

Range: The range of analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Accuracy: Accuracy of analytical method is 'measure of how close the experimental value to the true value' accuracy of the method was determined by standard addition method. A known amount of standard drug is added to the fixed amount of pre-analysed injection solution. Percent recovery is calculated by comparing the area before and after addition of the standard drug. The standard addition method is performed at 50%, 100% and 150% level. The solutions are analysed in triplicate at each level as per the proposed method.

Precision: The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the

prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Limit of detection and Limit of Quantification: Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of Quantification (LOQ) is defined as the lowest concentration of analyte that can be quantified with a specified level of accuracy and precision. For this study, six replicates of the analyte at lowest concentration are measured and quantified.

Robustness: The robustness of the proposed method is estimated by changing flow rate of the mobile phase, pH of the buffer and composition of the mobile phase.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of method was determined by comparing the chromatograms of blank, standard and sample.

RESULTS AND DISCUSSION

The HPLC procedure was optimised with a view to develop an accurate assay method for simultaneous determination of ATEN, HCTZ and LOST in bulk and pharmaceutical dosage form by using column Hypersil C18 (250 x 4.6 mm internal diameter; 5 µm particle size) with mobile phase composition of acetonitrile: Potassium dihydrogen orthophosphate buffer in the ratio of 40:60 V/V. The mobile phase flow rate was 1.5 mL/min and both the components were measured with UV-Visible detector at 225 nm. Resulted in peaks with good shape and well resolved. The results of optimized HPLC conditions were shown in Table 1. The method was linear in the range of 50-150 µg/mL,12.5-37.5 µg/mL and 50-150 µg/mL for ATEN, HCTZ and LOST with correlation coefficient 1 and 0.999 for both HCTZ and LOST. Linear regression data for ATEN, HCTZ and LOST were given in Table 2, the linearity curves for ATEN, HCTZ and LOST were shown in Fig. 4, Fig. 5 and Fig. 6. The mean % recoveries were found to be 97.56, 97.72 and 98.06 for ATEN, HCTZ and LOST respectively. which indicate the method is accurate, the accuracy results were shown in Table 3. The % RSD for method precision was found to be 0.20, 0.36 and 0.31 for ATEN, HCTZ and LOST respectively. It indicates the method is precise. The precision results were shown in Table 4. The retention time of ATEN, HCTZ and LOST was 1.46, 2.21 and 3.30 min. The number of theoretical plates calculated was 2791 for ATEN, 4745 for HCTZ and 6052 for LOST. Symmetry factor was 1.32 for ATEN,1.37 for HCTZ and 1.18 for LOST, which indicates efficient performance of the column, results of system suitability study were shown in the Table 5. The LOD for the ATEN, HCTZ and LOST were found to be 0.613 µg/mL, 0.158 µg/mL and 0.79 µg/mL respectively. The LOQ for the ATEN, HCTZ and LOST were found to be 1.85 µg/mL, 0.479 µg/mL and 2.42 µg/mL respectively, which indicates the sensitivity of the method. Results of study was shown in the Table 6. Validated RP-HPLC method was applied for the determination of ATEN, HCTZ and LOST in commercial tablet formulation that was obtained by injected 3 replicates of the sample solutions. The amounts of ATEN, HCTZ and LOST estimated were found to 99.84%, 98.16 and 97.14 % respectively. The results are shown in Table 7. Typical chromatogram of standard ATEN, HCTZ and LOST was shown in Fig. 7. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in pharmaceutical formulations did not interfere with the estimation of the drugs by the proposed method, which indicate method was specific. Results of specificity study was shown in Fig.8 The typical variations studied under this parameter were mobile phase composition and detection wavelength. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust.

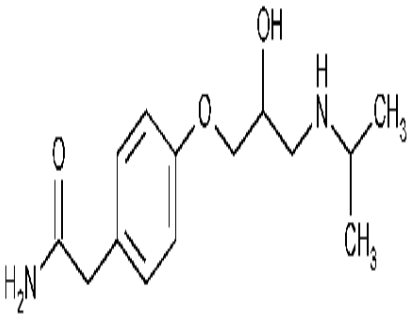


Fig. 1: Chemical Structure of Atenolol

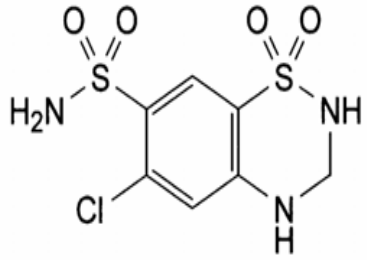


Fig. 2: Chemical Structure of Hydrochlorothiazide

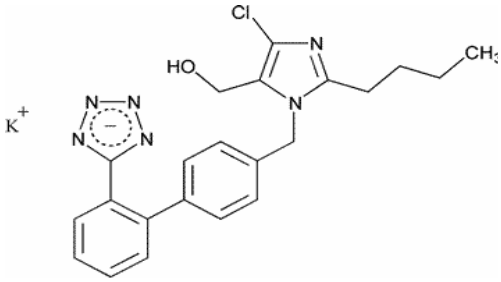


Fig.3: Chemical Structure of Losartan potassium

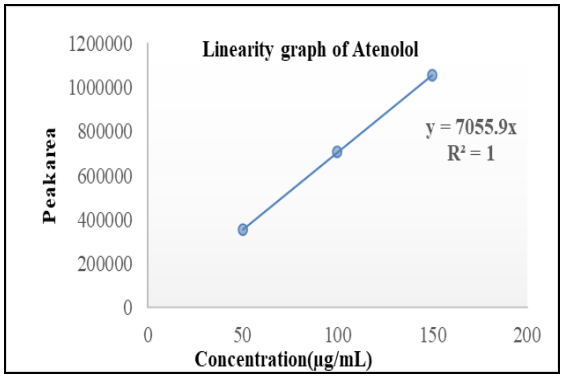


Fig. 4: Linearity graph of Atenolol

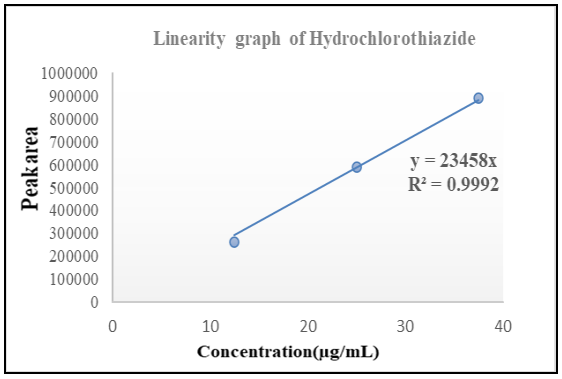


Fig.5: Linearity graph of Hydrochlorothiazide

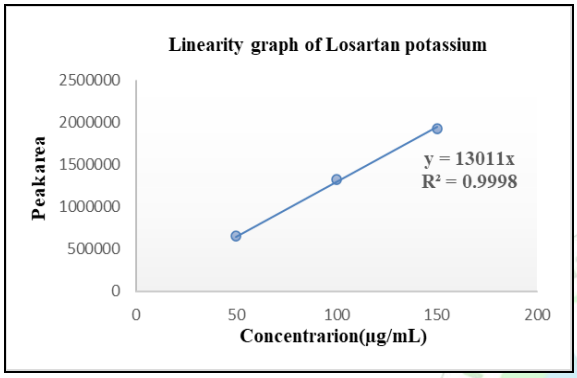


Fig. 6: Linearity graph of Losartan potassium

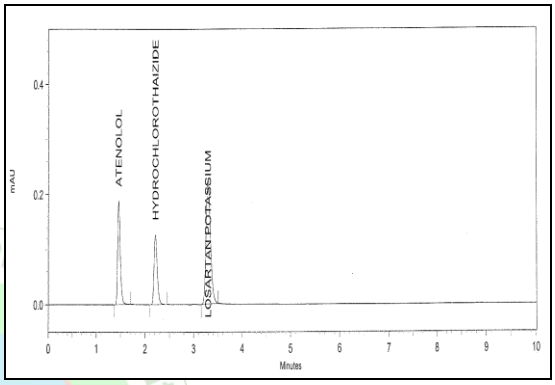
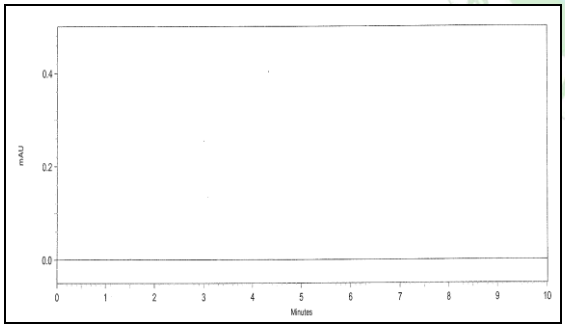
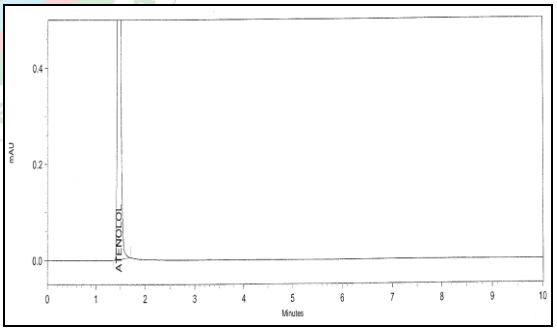


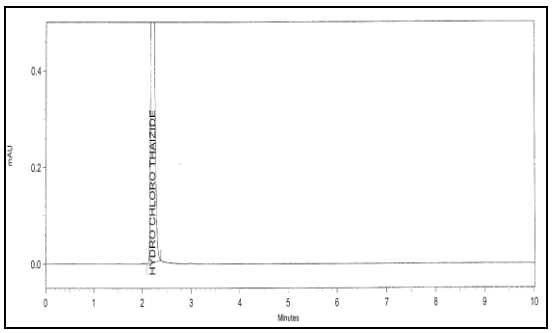
Fig. 7: Chromatogram of Atenolol, Hydrochlorothiazide and Losartan potassium

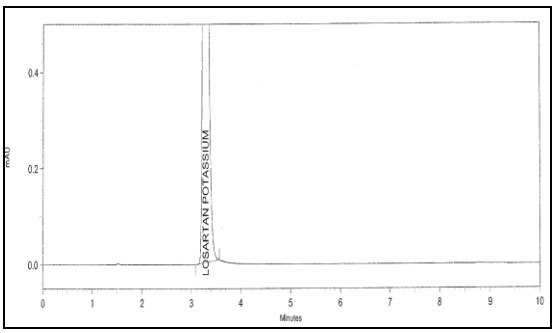


(a)



(b)





(c)

Figure. 8: Chromatograms of a). blank, b). Atenolol, c). Hydrochlorothiazide and d). Losartan potassium

Table 1: Optimized Chromatographic Conditions of the method

Parameter	Condition
Instrument	A Quaternary Gradient WATERS HPLC e2695 with QCL-034 Software with UV-Visible Detector (WATERS 2489), PUMP (LC-20AT) and (LC-20ATvp)
Mobile phase	Acetonitrile: Potassium dihydrogen orthophosphate buffer (40:60 % v/v)
pH	5.6 (Adjusted with dil. ortho phosphoric acid)
Flow rate	1.5 mL/min
Column	Hypersil, C_{18} Column (250 x 4.6 mm; 5 µm)
Column temperature	40° C
Injection volume	20 µL
Detection wave length	225 nm
Run time	10 min
Retention time	Atenolol- 1.46 min, Hydrochlorothiazide- 2.21 min and Losartan potassium- 3.30 min

Table 2: Linearity results of the method

S. No.	Atenolol		Hydrochlorothiazide		Losartan potassium	
1.	**Concentration (µg/mL)**	**Area**	**Concentration (µg/mL)**	**Area**	**Concentration (µg/mL)**	**Area**
2.	50	352595	12.5	263257	50	663311
3.	100	706492	25.0	587785	100	1328935
4.	150	1057851	37.5	888775	150	1928843

Table 3: Accuracy result of the method

Drug	% concentration (at specification level)	Amount added (µg/mL)	Amount found (µg/mL)	% recovery	Mean % recovery
Atenolol	50 %	50	47.80	95.61	97.564
	100 %	100	97.82	97.82	
	150 %	150	148.68	99.26	
Hydrochlorothiazide	50 %	12.5	12.19	97.54	97.723
	100 %	25.0	24.06	96.24	
	150 %	37.5	37.27	99.39	
Losartan potassium	50 %	50	49.28	98.56	98.062
	100 %	100	96.63	96.63	
	150 %	150	148.51	99.00	

Table 4: Method Precision results of the method

Parameter	Peak Area of Atenolol	Peak Area of Hydrochlorothiazide	Peak Area of Losartan potassium
Injection 1	655016	587304	1190777
Injection 2	657178	589922	1194393
Injection 3	657506	591760	1198086
Injection 4	654531	586769	1189894
Injection 5	657138	591695	1198123
Injection 6	655110	588375	1191145
Average	656079.83	589704.2	1193736.3
Std. Dev.	1328.97	2157.49	3710.25
% RSD	0.20	0.36	0.31

Table 5: Results of System Suitability study

S. No.	Parameters	Atenolol	Hydrochlorothiazide	Losartan potassium
1.	Retention times (min)	1.4	2.4	3.5
2.	Theoretical plates(N)	2791	4745	6052
3.	Tailing factor	1.32	1.37	1.18
4.	Resolution (Rs)	-	6.2	7.3

Table 6: Results of LOD and LOQ

S.No.	Parameter	Atenolol	Hydrochlorothiazide	Losartan potassium
1.	LOD	0.613 µg/mL	0.158 µg/mL	0.79 µg/mL
2.	LOQ	1.85 µg/mL	0.479 µg/mL	2.42 µg/mL

Table 7: Results of marketed formulation (Assay)

S.No.	Tablet dosage form	Drug	Label claim (mg/tablet)	Amount found (mg/tablet)	% Assay
1.	REPALOL H	Atenolol	50	49.92	99.84
2.		Hydrochlorothiazide	12.5	12.27	98.16
3.		Losartan potassium	50	48.57	97.14

CONCLUSION

Proposed Study describes new HPLC method for the simultaneous estimation of Atenolol, Hydrochlorothiazide and Losartan potassium in bulk samples and its pharmaceutical dosage form. The method was validated and found to be simple, accurate, precise and robust. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of estimation of ATEN, HCTZ and LOST in regular quality control testing laboratories.

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CONFLICTS OF INTEREST -Nil-

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