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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF RP HPLC MATHOD FOR THE SIMULTANEOUS ESTIMATION OF ATENOLOL, LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE IN TABLET FORMULATION

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Abstract: A new simple, rapid, precise and accurate assay method was developed for simultaneous estimation of Atenolol, Losartan and Hydrochlorthiazide in pure form and tablet form. The analytes were separated by RP HPLC on a RP-Purosnosphere C18 column (5 µm, 4.6mm* 250 mm).The mobile phase was methanol:water (77:23, v/v) at 1.1 mL/min flow rate satisfactorily resolve the tertiary mixture. The UV detector was operated at 230 nm for the determination of all the drugs. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 20-120 µg/ml for Atenolol and Losartan while 5-30 µg/ml for Hydrochlorthiazide with a R^2 0.9999, 0.9998 and 0.9990 values respectively. The optimized methods proved to be specific, robust and accurate for the quality control of drugs in bulk drug and pharmaceutical formulations.

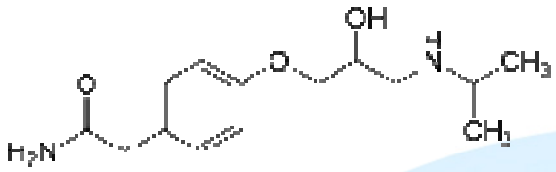
Key words: Atenolol, Losartan, Hydrochlorthiazide, Method Validation, RP HPLC,

INTRODUCTION:

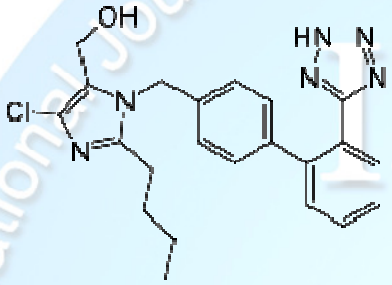
Drugs play a vital role in the progress of human civilization by curing diseases. Analytical chemistry is divided into two branches qualitative and quantitative[1]. Today a majority of the drugs used are of synthetic origin. These are produced in bulk and used for their therapeutic effects in pharmaceutical formulations. Pharmaceutical product quality is of vital importance for patient safety. Pharmaceutical analysis is the branch of pharmacy that is responsible for developing sensitive, reliable and accurate methods for the estimation of drugs in pharmaceutical dosage forms and biological fluids.[2] Atenolol (ATN) is a β_1 receptor specific antagonist, chemically (RS)-4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide[3], Losartan is (LOS), 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2'-(1*H*-te-trazol-5-yl)(biphenyl-4-yl)methyl]imidazole, potassium salt[4], while Hydrochlorthiazide (HCTZ) is a diuretic agent, chemically described as a 6-Chloro-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide[5]. Atenolol, Losartan in combination with hydrochlorthiazide is used in treatment of hypertension. Several methods are available in the literature for the determination of ATN, LOS and HCTZ most of these methods are for the determination of ATN, LOS or HCTZ separately, or in combination with other drug. Analytical methods reported for quantitative determination of ATN individually in pharmaceutical formulations or biological fluids are HPLC[6,7,8] and UV[9,10,11]. Analytical methods reported for quantitative determination of LOS individually inpharmaceutical formulations or biological fluids are HPLC[12, 13] and UV[14,15]. Analytical methods reported for quantitative determination of HCTZ individually in pharmaceutical formulations or biological fluids are HPLC[16,17], UV[18] and HPTLC[19,20,21].

These drugs are used in combination therapy not only because blood pressure control is often inadequate using monotherapy but also because combination therapy can simplify dosing regimens, improve compliance, decrease side effects and reduce cost. Literature survey revealed that very few methods are reported for determination of Atenolol, Losartan and Hydrochlorothiazide in pharmaceutical formulations. Therefore it was thought worthwhile to develop simple, precise and robust analytical method for the same.

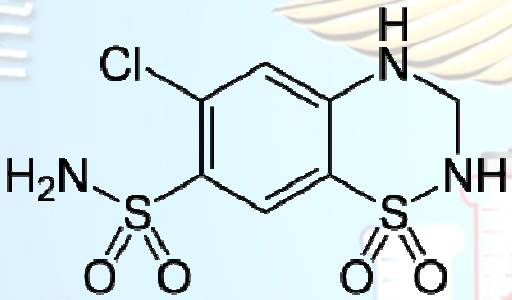
Figure 1 Chemical structures of Atenolol, Losartan and Hydrochlorthiazide



Atenolol



Losartan



Hydrochlorthiazide

EXPERIMENTAL

Chemicals and reagents

Atenolol and Losartan potassium were obtained as generous gift sample from Unichem Laboratories Ltd. (Sikkim) and Hydrochlorothiazide from Piramal Healthcare Pvt. ltd (Mahad). Commercial pharmaceutical preparation Repalol® H tablets, manufactured by Sun Pharma. Ltd., containing Atenolol (ATN) 50mg, Losartan (LOS) 50 mg and Hydrochlorthiazide (HCTZ) 12.5mg was collected from local market. Acetonitrile, methanol and water used were of HPLC grade (Qualigens Fine Chemicals, Mumbai, India). Ortho-phosphoric acid was AR grade (Qualigens Fine Chemicals, Mumbai, India). A 0.2 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Apparatus

The chromatographic system (Systronics Corporation, India) consisted of LC 8600 a prominence solvent delivery module, a manual injector with a 20 µL fixed loop and a UV-visible detector. The separation was performed on a Hibar® (Merk, Germany) RP-Purosphere Star C18 column (5 µm, 4.6mm* 250 mm) at an ambient temperature. Chromatographic data were recorded and processed using Chemitochrom 2000 software. An Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase.Shimadzu UV 1800 double beam UV visible spectrophotometer and Sansui-vibra DJ-150S-S electronic balance were used for Spectrophotometric and weighing purposes respectively.

Chromatography Conditions

Chromatographic separations of active (ATN, LOS and HCZ) substances were obtained by using Hibar® (Merk, Germany) RP-Purosphere Star C18 column (5 µm, 4.6mm* 250 mm),. Mobile phase methanol:water (77:23 v/v) (PH 3.0 was adjusted with 10% O-Phosphoric acid) was prepared, filtered through a 0.2 µm nylon filter and degassed for 5 min in an ultrasonicator. The mobile phase was pumped through the column at flow rate of 1.1 mL/min^{-1}. Analyses were carried out at ambient temperature with detection at 230 nm. The injection volume was 20 µL and each analysis required 12 min.

Standard Solutions

Stock standard solutions of ATN 1 mg/mL, LOS 1 mg/mL and HCTZ 1 mg/mL were prepared by dissolving 50 mg ATN, 50 mg LOS standard and 50 mg HCTZ standard in 50 mL methanol. Working standard solutions of ATN 0.1 mg/mL, LOS 0.1 mg/mL and HCTZ 0.1 mg/mL were prepared by diluting suitable aliquots of corresponding stock solutions with mobile phase.

Sample Solution

Twenty Repalol® H Tablets containing ATN (50 mg) and LOS (50 mg) HCTZ (12.5 mg) were weighed and ground to fine powder. A quantity of sample equivalent to ATN (50 mg), LOS (50 mg) and HCTZ (12.5 mg) was transferred into 100 mL volumetric flask containing methanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through 0.45µm nylon membrane filter. This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The response of solution was measured at 230 nm and quantification of ATN, LOS and HCTZ was done by using present HPLC method. Typical chromatogram of final resultant formulation solution was shown in (Fig. 1).

Validation of Proposed Method

Calibration curve (linearity)

Accurately measured aliquots of working standard solutions equivalent to 20-120 µgm/mL ATN, 20-120 µg/mL LOS and 5-30 µg/mL HCTZ were transferred to series of 10 mL volumetric flasks and the contents of the flasks were diluted to volume with mobile phase. A 20 µL aliquot of each solution was injected in triplicate into the liquid chromatography. The conditions including the flow rate of mobile phase at 1.1 mL/min, detection at 230 nm and run time program for 12 min, were adjusted. A calibration curve for each drug was obtained by plotting area under the peak versus concentration. The graphs of area vs concentration were recorded for all the drugs and are shown in (Fig. 2, 3 and 4).

Accuracy (% recovery)

Recovery studies were carried out by adding a known amount of pure drugs ATN, LOS and HCTZ to a pre analyzed sample solution. These studies were carried out by spiking 80%, 100% and 120% respective drug. The recovery studies showed that the results were within acceptable limits, above 99% and below 101%. The results are given in (Table 2)

Method precision (repeatability)

The precision of the developed method was assessed in terms of repeatability, intraday and inter-day precision by analyzing six replicate standard samples. The % R.S.D. values of the results corresponding to the peak area and retention time were expressed for intra-day precision and on 3 days for inter-day precision.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 5 times on the same day and on 5 different days for present method. The results are reported in terms of relative standard deviation (RSD).

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of the drug werecalculated using the equations according to International Conference on Harmonization (ICH) guidelines

Robustness

Robustness of the method was determined by making slight changes in chromatographic conditions. Effect of % of methanol (76, 77 and 78%) in mobile phase on the retention time and slight changes in flow rate were applied as variable parameters. Flow rate varied at three levels (-1, 0, 1). One factor at the time was changed to estimate the effect. Thus standard solution at varied pH (pH 2.9, 3.0 and 3.1) three pH levels was performed

Specificity

Specificity is the ability of theanalytical method to measure analyte response in presence of interferences including degradation products and related substances. Specificity was checked by determining ATN, LOS and HCTZ in laboratory prepared binary mixture and in binary mixture containing different degradation products.

System suitability Test

In the systemsuitability test tertiary solution of 50 µg/ml of ATN, 50 µg/ml of LOS and 12.5 µg/ml of HCTZ (n=6) was prepared and injected. Then the system suitability parameters like retention time, theoretical plates, tailing factor and resolution were calculated from the chromatogram.

Table 1. Regression analysis of the calibration curves for Atenolol, Losartan and Hydrochlorthiazide in the proposed HPLC Method

Parameter	Atenolol	Losartan	Hydrochlorthiazide
Linearity Range (µg/mL)	20-120	20-120	5-30
Detection Wavelength (nm)	230		
Slope ± SD	2.735	11.61	9.93935
Intercept ± SD	0.4307	4.8693	0.0826
Correlation coefficient	0.9999	0.9998	0.999

SD- Standard deviation, Mean of three determinations

Table 2. Summary of the validation parameters for the proposed HPLC method

Parameter	Atenolol	Losartan	Hydrochlorthiazide
LOD	1.5257µg /mL	1.9102µg /mL	1.0137µg /mL
LOQ	4.6235µg /mL	5.7886µg /mL	3.0719µg /mL
Accuracy,%	99.72±0.64	99.72±0.64	99.44±0.17
Repeatability (%RSD, n = 5)	0.168	0.379	0.458
Precision (RSD, %)			
Interday, n = 3	99.25 (0.252)	99.97 (0.538)	99.68 (0.375)
Intraday, n = 3	99.01 (0.259)	99.20 (0.352)	99.52 (0.270)

LOD = Limit of detection; LOQ = Limit of quantification; RSD = Relative standard deviation.

Table 3. Assay results for the combined dosage form using the proposed HPLC method

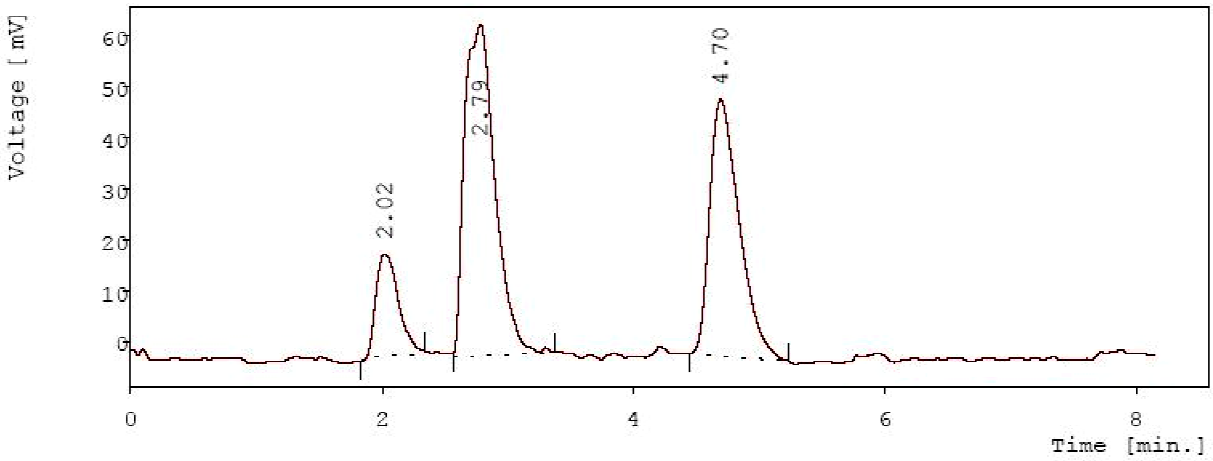
Formulation	Atenolol	Losartan	Hydrochlorothiazide
Repalol*H	99.54±0.931	99.63±0.538	100.85±0.534

SD= Standard deviation, 5 determinations.

Table No. 4. System suitability test parameters for ATN, LOS and HCTZ for the proposed HPLC method

System Suitability Parameters	Proposed Method		
	ATN	HCTZ	LOS
Retention Time (t_R)	2.027	2.750	4.683
Capacity Factor (k)	0.19	0.11	0.88
Theoretical Plate Number (N)	2927	5829	16503
Asymmetry factor	2.0	1.373	1.959
Resolution Factor (R)	0	2.43	4.190

Figure-1 Typical liquid chromatogram obtained for a 20 µL injection of a synthetic tertiary mixture of ATN, LOS and HCTZ



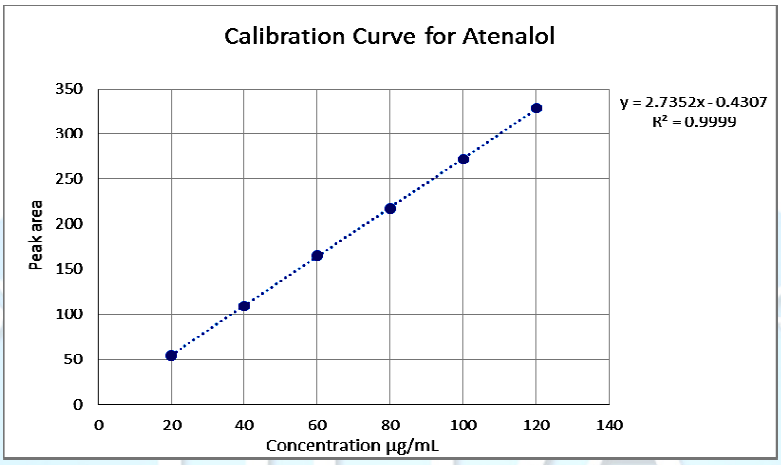


Fig. 2: Calibration Curve for Atenalol

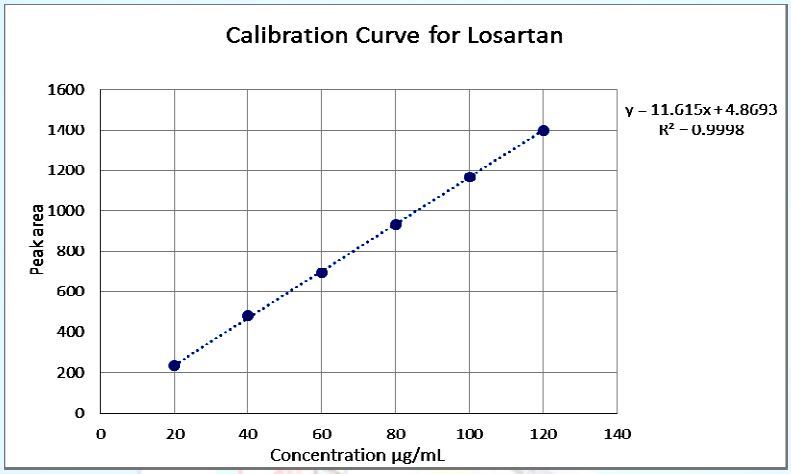


Fig. 3: Calibration Curve for Losartan

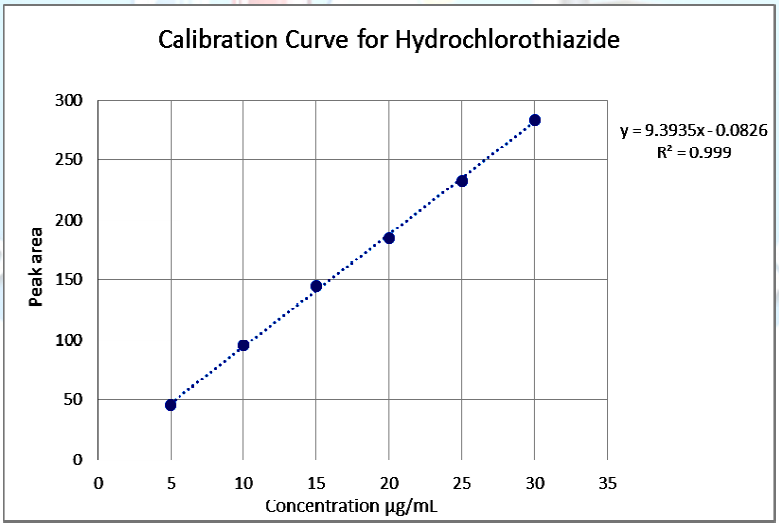


Fig.4: Calibration Curve for Hydrochlorothiazide

RESULTS AND DISCUSSION

The absorption spectra of ATN, LOS and HCTZ greatly overlap; so conventional determination of these compounds in mixture is not possible. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for ATN, LOS and HCTZ were obtained with a mobile phase consisting of methanol: water (77:23 v/v), pH 3.0 adjusted using 10% *o*-phosphoric acid. Quantification of the drugs was performed at 230 nm. Resolution of the components with clear baseline separation was obtained.

Validation of the Proposed Method

Linearity

Linear correlation was obtained betweenpeak areas and concentrations of ATN, LOS and HCTZ in range of 20–120, 20–120 and 5–30 µg/mL, respectively. The linearity of calibration curves was found to be acceptable over the concentration ranges of 20-120 µg/ml for Atenolol and Losartan while 5-30 µg/ml for Hydrochlorthiazide with a R^2 0.9999, 0.9998 and 0.9990 values respectively.

(Table- 1, Fig- 2, 3 and 4). The results show that good correlation existed between the peak area and concentration of the analysts.

Accuracy

The recovery experiments wereperformed by the standard addition method. The recoveries obtained were 99.72, 99.72 and 99.44% for ATN, LOS and HCTZ, respectively (Table 2). The high values indicate that the method was accurate.

Method precision

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit 0.168, 0.379 and 0.458 i.e. % RSD below 2.0 indicating that the method is reproducible. The results are shown in (Table No.2)

Intermediate precision

The intraday RSD values for ATN, LOS and HCTZ were 0.252-0.259, 0.347-0.352 and 0.244-0.270%, respectively. The interday RSD values for ATN, LOS and HCTZ were 0.136–0.152, 0.536-0.538 and 0.374–0.375%, respectively. The % RSD (< 2%) values indicate that the method was sufficiently precise (Table 2).

LOD and LOQ

LOD values for ATN, LOS and HCTZwere found to be 1.5257 µg/mL, 1.9102 µg /mL and 1.0137 µg /mL, respectively. LOQ values for ATN, LOS and HCTZ were found to be 4.6235 µg /mL, 5.7886 µg /mL and 3.0719 µg /mL, respectively (Table 2). These data showed that the method was sensitive enough for the determination of ATN, LOS and HCTZ.

Robustness

The method was found to be robust with no significant changes on test result upon change of analytical conditions like different flow rate, % methanol in mobile phase and pH of mobile phase with the standard deviation was found to be bellow 1 and % RSD is less than 2 for all results. It was found that under small deliberate changes of chromatographic factors, there was no considerable change in under study parameters.

System Suitability Test

A tertiary solution of 50µg/mL of ATN, 50µg/mL LOS and 12.5 µg/mL of HCTZ (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated. The results (Table 4) obtained from system suitability tests are in agreement with the official requirements.

CONCLUSIONS

The proposed LC method presented in this paper has advantages of simplicity, accuracy, precision and convenience for separation and quantitation of ATN, LOS and HCTZ in combination and can be used for the assay of their respective dosage form. Moreover, the proposed LC method is a stability indicating assay method that can determine ATN, LOS and HCTZ in presence of their degradation products. Thus, the proposed LC method can be used for the quality control of ATN, LOS and HCTZ in typical laboratories.

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