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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATENOLOL IN COMBINATION WITH HYDROCHLOROTHIAZIDE AND LOSARTAN POTASSIUM IN BULK AND TABLET DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

Two sensitive, precise, accurate and simple methods has been developed and validated for the analysis of Atenolol, in combination with Hydrochlorothiazide and Losartan potassium in tablet dasage form. Method A involved chromatographic estimation of Atenolol in combination with Hydrochlorothiazide and Losartan potassium in tablet dasage form by RP-HPLC. Chromatographic resolution was achieved on a reverse-phase Zorbax SB-C18 (150 x 4.6 mm), 3.5µm column using acetonitrile: water: 0.05mM sodium dihydrogen ortho phosphate (pH 2.5) in the ratio of (40:10:50) as mobile phase with a flow rate of 0.7mL/min and isocratic elution with a total run time of 8 minutes. The retention time of Atenolol, Hydrochlorothiazide, Losartan potassium was found to be 1.921, 2.630 and 5.061 respectively. Detection of the multi compounds was carried out at 210nm. The peaks of atenlol, hydrochlorothiazide and losartan potassium were well separated . The Calibration curves were linear over studies ranges with correlation co-efficient found between the range of 0.99 to 1.00. The proposed method is accurate with 99.8% recovery for atenolol, 100.9% recovery for hydrochlorothiazide and 99.6% recovery for losartan potassium and precise (% RSD < 0.5).

Key words: Atenolol, Hydrochlorothiazide, Losartan potassium, HPLC..

INTRODUCTION

Atenolol[1] is chemically1 2-{4-[2-hydroxy-3-(propan-2- ylamino) propoxy] phenyl} acetamide. It is a β1 receptor specific antagonist, a drug belonging to the group of β-blockers, a class of drugs used primarily in cardiovascular diseases.

Hydrochlorothiazide[1] (HCTZ) is chemically1 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide. HCTZ is a popular diuretic drug of the thiazide class. It is often used in the treatment of hypertension, congestive heart failure symptomatic edema and in the prevention of kidneystones. Losartan [1] is chemically1 (2-butyl-4-chloro-1- {[2'-(1*H*-tetrazol-5-yl) biphenyl-4-yl] methyl}-1*H*imidazol- 5-yl) methanol. Losartan is an angiotensin II receptor antagonist drug used mainly to treat high BP (hypertension) [2-4].

Several analytical techniques have been reported for the analysis of individual compounds and with different combinations. Since some of UV and HPLC analytical techniques were available for the analysis of Atenolol, Hydrochlorothiazide and Losartan potassium wiyh different combinations.The present developed methods were relatively simple, rapid and highly sensitive and validated as stated in ICH guidelines in the analysis of the multi components of interest and it can be used for routine Quality control analysis in laboratories [5].

EXPERIMENTAL

Materials

Atenolol (purity: 98.91%), Hydrochlorothiazide (purity: 99.97%) and Losartan potassium (purity: 99.81%) were obtained from Sunpharma laboratories (India) and Micro labs (Bangalore, India). Acetonitrile was of HPLC grade and obtained from E.merck (Mumbai, India) and all other chemicals used were of analytical grade. Purified

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water from Milli-Q-system (Millipore, Bangalore, India) was used throughout the analysis. Methanol was used as a solvent for UV analysis obtained from E.merck (Mumbai, India) [6].

Chromatographic System and Conditions

HPLC Chromatographic separation was performed on a Shimadzu integrated high performance liquid chromatographic system was used for this experiment. This system equipped with quaternary gradient pump, UV-VIS detector W2487, Column Oven and programmable auto sampler controlled by Empower2 software. Best HPLC separation was carried out using reverse phase Zorbax SB-C18 (150 × 4.6 mm) 3.5µ column of , using acetonitrile: water : 0.05M sodium dihydrogen orthophosphate (pH 2.5) in the ratio (40:10 :50) as mobile phase at a flow rate of 0.7 ml/min and detection and detection carried out at 210 nm. The mobile phase was filtered through a 0.45 µm membrane filter (Millipore) [7].

Mobile phase Preparation

Mix a mixture of above buffer (Sodium Phosphate- P^H 2.5**)** 500 ml (50%) and 400 ml of Acetonitrile HPLC (40%) and 100 ml of Water HPLC (10%) and degased in ultrasonic water bath for 5 minutes. Filterred through 0.45 µ filter paper under the vacuum filtration [8].

Standard Preparation

Accurately weigh and transfer 50 mg of Atenolol and 12.5mg Hydrochlorothiazide and 50mg Losartan working standards into a 10ml of separate clean dry volumetric flask add about 7ml of Diluent to each flask and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.4ml of Atenolol, Hydrochlorothiazide & Losartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [9].

Sample Preparation

Twenty tablets, (Brand name : Repalol-H) each containing 50 mg Atenolol, 12.5 mg Hydrochlorothiazide and 50 mg Losartan potassium, were weighed and average weight (265.8 mg) was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.4ml of Atenolol, Hydrochlorothiazide& Losartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [10].

Method Validation

Method validation was performed according to the accepted guidelines . Briefly, precision, intra- and inter-day variations, linearity over a specified concentration range and accuracy (measured as percent recovery) were assessed. Linearity was evaluated over a concentration range of 100-300, 25-75 and 100-300 mcg/mL of Atenolol, Hydrochlorthiazide and Losartan Potassium respectively. at five different concentration levels. The repeatability study (Precision) (n = 5) was carried out showed a R.S.D. of Atenolol, Hydroclorothiazide and Losartan Potassium is 0.31%.0.27% and 0.37% respectively for the peak area ratios of each standard showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision(inter day), a study carried out by the same analyst working on two consecutive days (n = 5) indicated a R.S.D. of Atenolol, Hydroclorothiazide and Losartan Potassium is 0.19%, 0.27% and 0.13% respectively. Both values were far below 2%, the limit percentage set for the precision and indicated a good method precision. Accuracy were expressed in terms of percentage recoveries of Atenolol, Hydrochlorthiazide and Losartan Potassium in the real samples.

Specificity

The HPLC chromatogram recorded for the mixture of the drug excipients revealed no peak within a retention time range of 8.0 min. The results showed that the developed method was specific as none of the excipients interfered with the analytes of interest.

Stability

The stability of Atenolol, Hydrochlorthiazide and Losartan Potassium in standard and sample solutions was determined by storing the solutions at ambient temperature (20±1◦ C) protected from light. The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 72 h, as during this time the results did not decrease below 97%. This denotes that standard and sample solutions are stable for at least 3 days at ambient temperature.

System Suitability

The resolution factor between each Drug, in the developed method, was above 2. The % R.S.D. of peak area ratios of each Drug and retention time for each drug were within 2% indicating the suitability of the system (Table. 5). These results indicate the applicability of this method to routine with no problems, its suitability being proved. The statistical evaluation of the proposed method revealed its good linearity, reproducibility and its validation for different parameters and led us to the conclusion that it could be used for the rapid and reliable determination of Atenolol, Hydrochlorthiazide and Losartan Potassium in pharmaceutical forms.

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Assay of Tablets

The validated method was applied for the assay of commercial tablets containing 50mg, of Atenolol, 12.5 mg of Hydrochlorthiazide and 50 mg of Losartan Potassium (Repalol-H) each sample was analysed in triplicate after extracting the drug as mentioned in assay sample preparation of the experimental section and injections were carried out in triplicate. Shows an HPLC chromatogram of pharmaceutical tablets. None of the tablets ingredients interfered with the analyte peak

RESULTS AND DISCUSSION

Table 1. Assay Results of Tablet Formulation

Serial no	Drug name	Lable claim *(Mg)*	Amount Found (mg)	% Assay Found
1	Atenolol	50	0.1	100
2	HCTZ	12.5	0.1	100
3	Losartan	50	0.996	99.6

Table 2. Results for Linearity

Drug name	Linearity Level	Concentration	Area
	I	100ppm	545646
	II	150ppm	803903
Atenolol	III	200ppm	1106609
	IV	250ppm	1337748
	V	300ppm	1589891
	Correlation Coefficient		0.999
	I	25ppm	482358
	II	37.5ppm	693785
Hydrochlorothiazide	III	50ppm	944321
	IV	62.5ppm	1126295
	V	75ppm	1343666
	Correlation Coefficient		0.999
	I	100ppm	2399999
	II	150ppm	3459198
Losartan potassium	III	200ppm	4705386
	IV	250ppm	5650058
	V	300ppm	6726138
	Correlation Coefficient		0.999

Table 3. Results for Accuracy

Drug name	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	%Mean Recovery
	50%	1054725	25	25.1	100.1%	
ATN	100%	2094717	50	49.9	99.8%	99.8%
	150%	3136769	75	74.7	99.6%	
	50%	910370	6.34	6.34	101.4%	
HCTZ	100%	1792328	12.4	12.4	99.9%	100.9%
	150%	2730808	19.0	19.0	101.4%	
	50%	4540880	25	25.1	100.5%	
LOS	100%	9009431	50	49.8	99.7%	99.6%
	150%	13336102	75	74.1	98.8%	

Table 4. Results for System Suitability parameters

Parameters	Atenolol	HCTZ	Losartan
Theoretical plate/meter	2484.0	2765.4	2170.4
Asymmetricfactor/ tailindg factor	1.7	1.7	1.5

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LOD(ng/ml)	2.93	2.95	3.04
LOQ(ng/ml)	9.95	10	10.1

Table 5. Results for Precision(Repeatability) and Intermediate Precision(Ruggedness)

Injection	**Area**					
	Precision(Repeatability)			**Intermediate Precision(Ruggedness)**		
	Atenolol	**HCTZ**	**Losartan potassium**	**Atenolol**	**HCTZ**	**Losartan potassium**
Injection-1	1101705	944009	944009	1141296	954546	4827682
Injection-2	1103003	939851	939851	1144594	953426	4820562
Injection-3	1100367	935551	935551	1144293	952159	4836987
Injection-4	1103198	938507	938507	1145757	958064	4830499
Injection-5	1109194	937944	937944	1147058	957666	4832139
Average	1103494	939172	939172	1144600	955172	4829574
Standard Deviation	3383.5	3119.4	3119.4	2144.3	2603.0	6065.8
%RSD	0.31	0.33	0.33	0.19	0.27	0.13

Fig 1. Atenolol

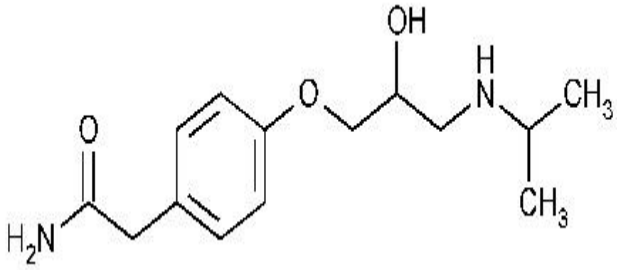


Fig 2. Hydrochlorothiazide

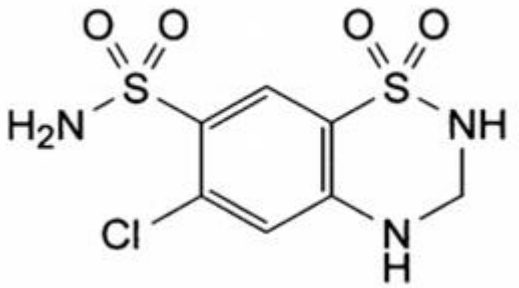


Fig 3. Losartan Potassium

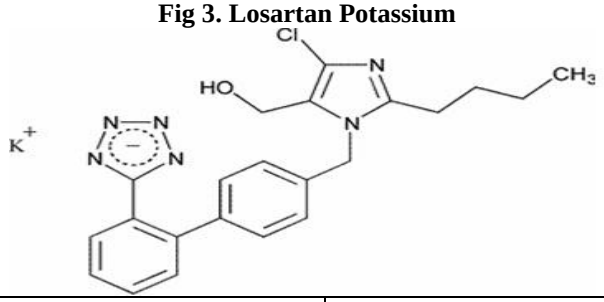
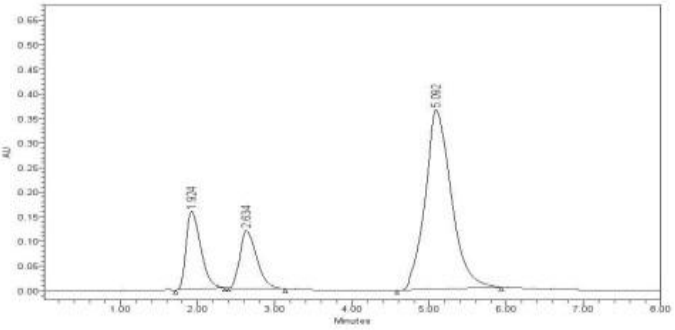
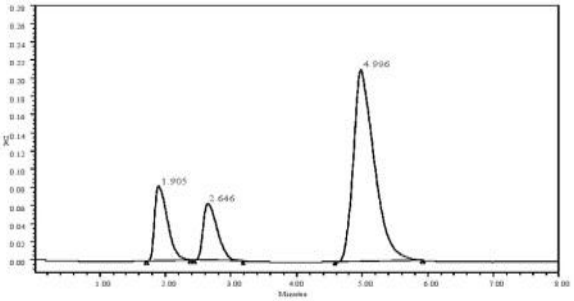


Fig 4. Standard Chromatogram:



	Name	Retention Time (min)	Area (µV*sec)
1	Atenolol	1.924	2094768
2	Hctz	2.634	1794299
3	Losartan	5.092	9008874

Fig 5. Sample Chromatogram



	Name	Retention Time (min)	Area (µV*sec)
1	Atenolol	1.905	1156140
2	Hctz	2.646	955751
3	Losartan	4.986	4834836

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Calibration curve for
Fig 10. Atenolol

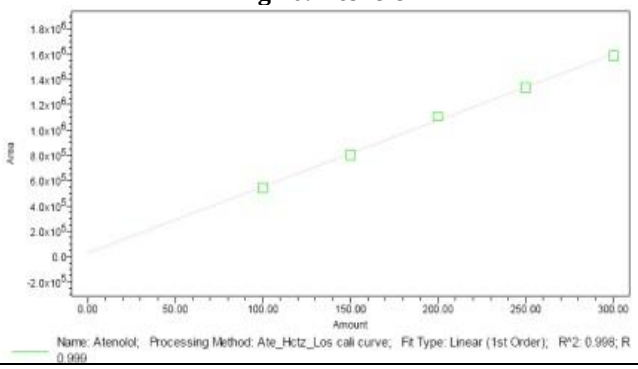


Fig 11. Hydroclorothiazide

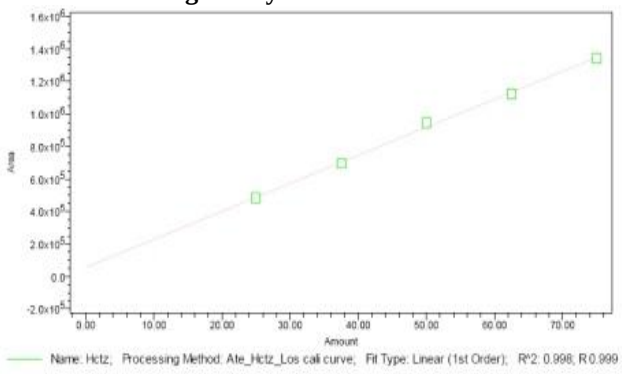
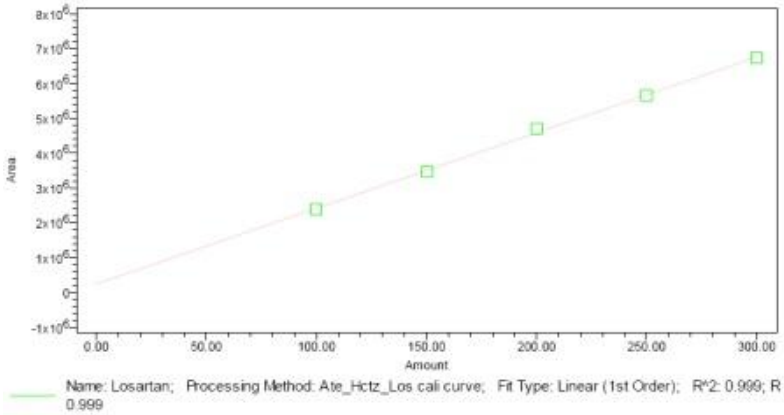


Fig 12. Losartan Potassium



CONCLUSION

Validated isocratic HPLC method has been developed for the determination of Atenolol in combination with Hydrochlorothiazide and Losartan potassium in bulk and tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. The chromatographic run time of 8.0 min/ml in HPLC allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of pharmaceutical dosage forms. The simplicity

of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS/MS or GC MS/MS that are complicated, costly and time consuming rather than a simple HPLC–UV method. The proposed methodcould be useful for the national quality control laboratories in developing countries.

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CONFLICT OF INTEREST

No interest

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