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ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.2, pp 880-884, April-June 2010

Development and Validation of New Method for Atenolol, Hydrochlorothiazide and Losartan potassium by RP-HPLC: Its Application to Routine Quality Control Analysis

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Abstract: Simple, rapid and sensitive UV, HPLC and Dissolution methods has been developed and validated for the analysis of Atenolol, Hydrochlorothiazide and Losartan potassium in tablet formulation. Best chromatographic resolution was achieved on a reverse-phase Phenomenex C_{18} column using acetonitrile: 50mM potassium dihydrogen ortho phosphate (pH 3.5) ratio 50:50 as mobile phase with a flow rate of 1mL/min and isocratic elution with a total run time of 14 minutes. Sulphadoxine was selected as internal standard. The retention time of Atenolol, Hydrochlorothiazide, Losartan potassium and Internal Standard was found to be 5.550, 3.280, 7.370 and 12.397 respectively. Detection of the multicompounds was carried out at 270nm. The present newly developed method was found to be accurate, precise and can be useful for routine Quality control analysis.
Keywords: Atenolol, Hydrochlorothiazide, Losartan potassium, UV, HPLC, Dissolution.

Introduction

Atenolol is chemically[1] 2-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} acetamide. It is a β_1 receptor specific antagonist, a dug belonging to the group of β-blockers, a class of drugs used primarily in cardiovascular diseases. Hydrochlorothiazide (HCT) is chemically[1] 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide. HCT 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 kidney stones. Losartan is chemically[1] (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). Many methods[2-15] have been reported on individual as well as simultaneous estimation on these drugs.

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The present method is relatively very simple, rapid and highly sensitive for multicomponent analysis of Atenolol, Hydrochlorothiazide and Losartan in bulk or in any formulation. Several analytical techniques have been reported for the analysis of individual compounds and with different combinations. Since no analytical techniques were available for the combined analysis of Atenolol, Hydrochlorothiazide and Losartan potassium the present method has been developed. The present method developed is relatively simple, rapid and highly sensitive and validated as stated in ICH guidelines in the analysis of the multicomponents of interest and it can be used for routine Quality control analysis in laboratories.

Experimental

Chemicals and Reagents

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

grade. Purified water from Milli-Q-system (Millipore, Bangalore, India) was used throughout the analysis.

Instrumentation and Analytical Conditions

HPLC Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector, Rheodyne 7725i injector with 50 µl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). Best HPLC separation was carried out using reverse phase C_{18} Princeton SPHER (250 × 4.6 mm) column of 5µ, using acetonitrile : 50 mM Potassium dihydrogen orthophosphate (pH 3.5) in the ratio (50 : 50) as mobile phase at a flow rate of 1.0 ml/min and detection and detection carried out at 270 nm. The mobile phase was filtered through a 0.45 µm membrane filter (Millipore®).

Preparation of Standard Solutions

Standard solutions were prepared (each) by accurately weighing 100.00 mg of each of the reference drug and transferred to 100.00 ml volumetric flask and dissolved in a mixture of acetonitrile: water (1:1v/v) to give a final concentration of 1 mg/mL, stored at 4°C and can be suitably diluted used for further analysis.

Preparation of Sample Solution

Twenty tablets, (Brand name : Tozaar-ATH) each containing 50 mg Atenolol, 12.5 mg Hydrochlorothiazide and 50 mg Losartan potassium, were weighed and average weight was calculated. One fourth of the average weight was accurately weighed and transferred to 100.00 ml volumetric flask and dissolved by sonication in a mixture of acetonitrile: water (1:1v/v) to give a final concentration of 1 mg/mL. The solution was stored at 4°C and suitably diluted for further analysis (Figure. 1).

Method Validation

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy specificity, short-term stability and system suitability. Atenolol Standard plots were constructed with six concentrations in the range of 5.0–60 mcg/mL prepared in triplicates to test linearity. Hydrochlorothiazide Standard plots were constructed with six concentrations in the range of 2.5–15 mcg/mL prepared in triplicates to test linearity. Losartan Potassium Standard plots were constructed with six concentrations in the range of 5–60 mcg/mL prepared in triplicates to test linearity The ratio of peak area signal of each drug to that of IS was plotted against the corresponding concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of each freshly prepared standard solution in the same equipment at a concentration 50 mcg/mL of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area ratios of each standard to that of IS were determined and precision was reported as % R.S.D. Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analysing samples of each drug at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of each drug recovered in the samples. Specificity was assessed by comparing the chromatograms obtained from sample of pharmaceutical preparation and standard solution with those obtained from excipients which take part in the commercial tablets and verifying the absence of interferences. Sample solution short-term stability was tested at ambient temperature (20±1° C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablets sample solutions, both solutions protected from light were reinjected after 24 and 48 h at ambient temperature and compared with freshly prepared solutions. A system suitability test was performed by six replicate injections of the standard solution at a concentration of 50 mcg/mL verifying IS/DI resolution >2; %R.S.D. of peak area ratios of each standard to that of IS ±2%; %R.S.D. of each peak retention time ±2%.

Results and Discussion

Validation of Methods

Linearity

Six point's calibration graphs were constructed covering a concentration range 10–60, 2.5-15 and 5-60 mcg/mL Atenolol, Hydrochlorthiazide and Losartan Potassium respectively. Three independent determinations were performed at each concentration. Linear relationships between the ratio of the peak area signal of each Standard to that of IS versus the corresponding drug concentration were observed. The standard deviations of the slope and intercept were low. The determination coefficient (r^2) exceeded 0.99 (Figure. 2).

Precision

The repeatability study (n = 6) carried out showed a R.S.D. of 0.858% for the peak area ratios of each standard to that of IS obtained, thus showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision, a study carried out by the same analyst working on two consecutive days (n = 3) indicated a R.S.D. of 0.744%. Both values were far below 5%, the limit percentage set for the precision and indicated a good method precision.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of Atenolol, Hydrochlorthiazide and Losartan Potassium in the real samples. These results are summarized in Table. 1.

Specificity

The HPLC chromatogram recorded for the mixture of the drug excipients revealed no peak within a retention time range of 15 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 containing IS 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 DI is stable in standard and sample solutions for at least 3 days at ambient temperature, protected from light and is compatible with IS.

System Suitability

The resolution factor between IS and each Drug, in the developed method, was above 2. The % R.S.D. of peak area ratios of each Drug to that of IS and retention times for both drug and IS were within 2% indicating the suitability of the system (Table. 2). 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.

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 (Tozaar-ATH) 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 (Figure. 1).

Figure 1. Sample Chromatogram

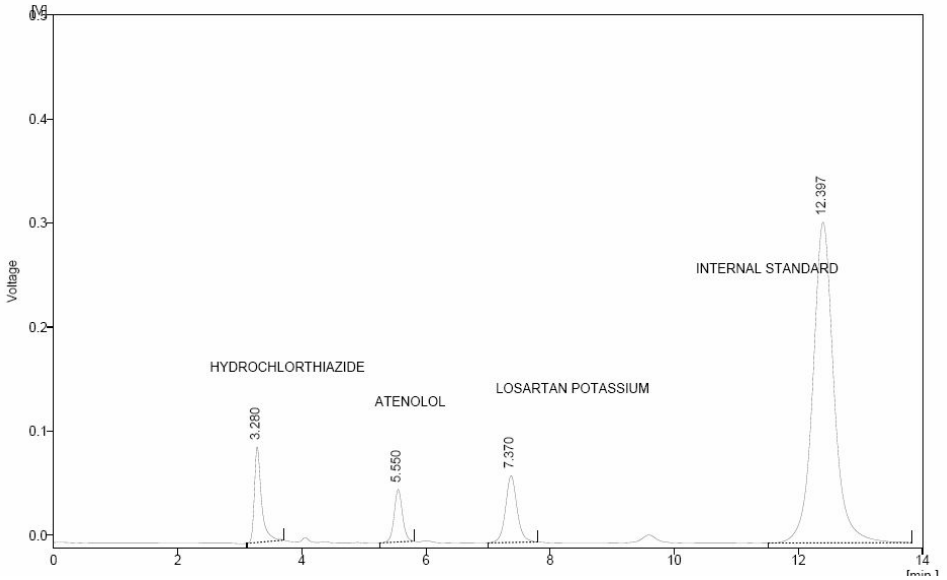

Figure 2. Calibration Curve

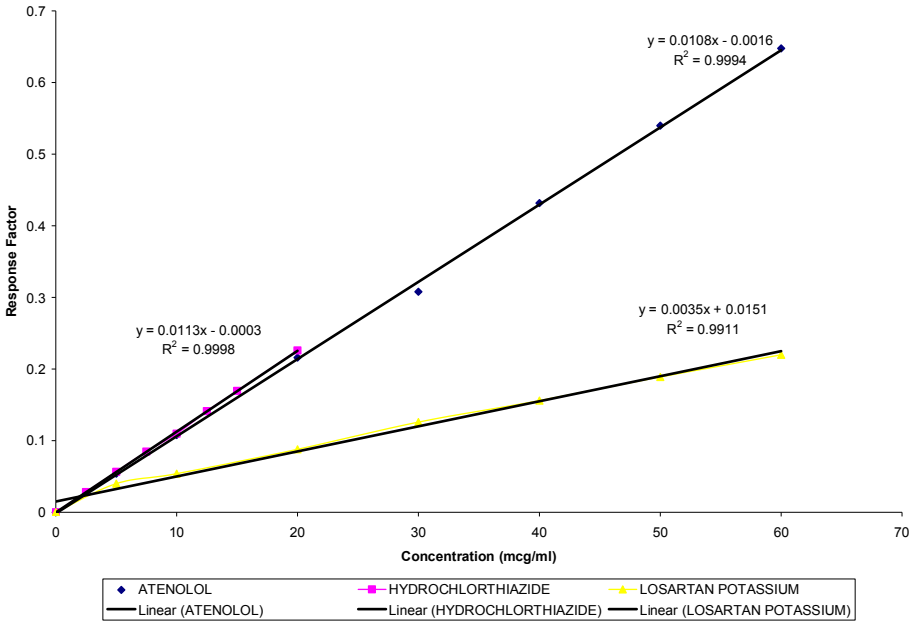

Table 1. Accuracy study for Tozaar-ATH (*n*=5)

Nominal Concentration (mcg/mL)	Observed Concentration (mcg/mL)	% C.V.
Atenolol		
5	4.5083±0.18	4.00
30	28.4405±0.68	2.40
60	58.3188±1.44	1.44
Hydrochlorthiazide		
2.5	2.299±0.10	4.25
7.5	7.3513±0.14	1.92
20	19.6017±0.41	2.10
Losartan Potassium		
5	4.5483±0.16	3.49
30	28.6405±1.43	5.00
60	57.9188±1.41	2.43

Table 2. System Suitability

Parameters	Atenolol	Hydrochlorthiazide	Losartan Potassium
Theoretical plate/meter	4525	5249	5390
Asymmetric factor	0.92	1.04	0.95
LOD (ng/ml)	10	5	7
LOQ (ng/ml)	30	15	20

Conclusion

Validated isocratic HPLC methods have been developed for the determination of Multicomponent dosage forms. The proposed methods are simple, rapid, accurate, precise and specific. Its chromatographic run time of 13 min 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. Considering the possible worldwide development of counterfeit Tozaar-ATH, the proposed method could be useful for the national quality control laboratories in developing countries.

Acknowledgements: We are grateful to my guide and to all my staffs who supported us.

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