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Simultaneous estimation of atenolol, hydrochlorothiazide, losartan and valsartan in the pharmaceutical dosage form

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Abstract

A simple, specific, accurate and economical isocratic reversed phase liquid chromatographic (RP-HPLC) method was developed and subsequently validated for the determination of atenolol, hydrochlorothiazide, losartan and valsartan. Separation was achieved with a Nucleodur 100 C–18 column having 250 x 4.6mm i.d. with 5µm particle size and potassium dihydrogen phosphate buffer adjusted to pH 3.0 using diluted ortho phosphoric acid and acetonitrile (50:50 v/v) with isocratic program as eluent at a constant flow rate of 1.0ml per min. UV detection was performed at 210nm. The retention time of atenolol, hydrochlorothiazide, losartan and valsartan was about 1.99min, 2.90min, 5.92min and 9.42min respectively. The proposed method was validated and successfully used for estimation of atenolol, hydrochlorothiazide, losartan and valsartan in the pharmaceutical dosage form.

Keywords: Validation, RP-HPLC, Atenolol (ATEN), Hydrochlorothiazide (HYD), Losartan (LOS) and Valsartan (VAL).

Introduction

The purpose of this method is to develop the (RP-HPLC) method for simultaneous determination of antihypertensive drugs - atenolol, hydrochlorothiazide, losartan and valsartan in pharmaceutical formulations. It is essential to have a validated, stability indicated or specific analytical method of analysis for the drug for which the drug delivery system is to be designed. Reversed phase liquid chromatographic technique (RP-HPLC) is one of the latest and most widely applied techniques for drug estimation. This method is simple, rapid and selective. The proposed method was validated and successfully used for determination of atenolol, hydrochlorothiazide, losartan and valsartan in the pharmaceutical dosage form. A literature survey regarding quantitative analysis of these drugs revealed that attempts were made to develop analytical methods for atenolol, hydrochlorothiazide, losartan and valsartan using spectrophotometry[1-5], HPTLC[6-7], HPLC[8-14], dosage clinical studies[15] and permeation studies[16]. This paper describes a new RP–HPLC method for the estimation of atenolol, hydrochlorothiazide, losartan and valsartan combination in mixture using simple mobile phase.

Atenolol is a competitive cardio selective β1- blocker. It does not have effect on β2- receptors except in high doses. Its cardio selectivity is dose related. Atenolol reduces resting and exercise induced heart rate as well as myocardial contractility. Resting cardiac output is increased to some extent. Atenolol reduces blood pressure (BP) and heart rate which results in reduced myocardial work and oxygen (O_2) requirement leading to improved exercise tolerance and reduced frequency and intensity of anginal attack.

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Hydrochlorothiazide is a thiazide diuretic often considered as the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism. Hydrochlorothiazide or HZT is a popular diuretic drug that acts by inhibiting the kidney's ability to retain water. This reduces the volume of the blood, decreasing peripheral vascular resistance. Losartan is an angiotensin II receptor antagonist. It acts as antihypertensive by blocking the actions of angiotensin II of renin-angiotensin aldosterone system. The drug and its active metabolite selectivity block**s** the vasoconstrictor and aldostrerone secreting effects of angiotensin II by selectively antagonising the binding of angiotensin II to AT_1 1 receptors. Neither losartan nor its metabolite inhibits ACE (kinase II). Valsartan is an angiotensin II receptor antagonist. It acts as an antihypertensive. Valsartan produces its blood pressure (BP) lowering effects by antagonizing angiotensin I induced vasoconstriction, aldosterone release, catecholamine release, water intake and hypertophic responses. Valsartan**,** a selective angiotensin II type 1 receptor (AT_1R) blocker, has beneficial effects in the cardiovascular system in part by its increase of nitric oxide (NO) bioavailability, yet the mechanisms are unclear.

Material and methods

The liquid chromatographic system consists of the following components: Agilent HPLC model (1200 series) containing quaternary pump, sample thermostat, column thermostat, thermostated auto sampler and variable wavelength programmable detector. Chromatographic analysis was performed using Chemstation software on a Nucleodur100 C–18 column with 250 x 4.6mm i.d. and 5 µm particle size. The Metter Telledo electronic balance (AX 105) was used for weighing purpose. Analytically pure ATEN, HYD, LOS and VAL were obtained as gift samples from my colleague Mr.Haresh Gurav (Asst.Manager QA/RA Dept. Nicholas Piramal India Ltd. Mumbai, India). Acetonitrile (E.Merck, Mumbai, India), water (TKA water purification system - Germany) were of HPLC grade, buffer potassium dihydrogen phosphate and ortho phosphoric acid (S.D. Fine Chemicals, Mumbai, India) were of analytical grade used for the preparation of mobile phase. Three commercial formulations each of ATEN (BP-NOL TABLETS, Elder Limited. Mumbai, India), HYD and LOS (COSART-H TABLETS, Cipla Limited. Mumbai, India) and VAL (VALENT CAPSULES, Lupin (Pinnacle) Limited. Mumbai, India) were selected from local market on random basis.

Preparation of reagent and solution

Potassium dihydrogen phosphate (KH_2PO_4) was weighed (1.36g) and dissolved in 1000 ml water. Finally the pH 3.0 was adjusted with diluted ortho phosphoric acid. The buffer solution was sonicated for about 5 to 10min. and filtered through 0.45µ filter paper. ATEN, HYD, LOS and VAL were weighed (50mg of each) and transferred to four separate 50ml of volumetric flasks separately and dissolved in water: acetonitrile (1:1) which gives 1000µg/ml of ATEN, HYD, LOS and VAL respectively. These stock solutions were further diluted with water: acetonitrile (1:1) to obtain final concentration of 200µg/ml of ATEN and 100µg/ml of HYD, LOS and VAL respectively. Appropriate aliquots of ATEN, HYD, LOS and VAL stock solutions were taken in 25.0ml volumetric flasks and diluted up to the mark with diluent water: acetonitrile (1:1) to obtain final concentration between 160µg/ml to 240µg/ml for ATEN and between 60µg / ml to 140µg/ml of HYD, LOS and VAL respectively.

Optimization of experimental condition

An isocratic reversed phase Nucleodur 100 C-18 column equilibrated with mobile phase buffer potassium dihydrogen phosphate (10 mmol) adjusted to pH 3.0 using diluted ortho phosphoric acid – acetonitrile (50:50) was used. Mobile phase flow rate was maintained at 1.0 ml per min. and effluents were monitored at 210nm. The sample was injected using a 10µl fixed loop and the total run time was 15.0min. The solutions were injected using 10µl fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peaks versus concentrations. Correlation coefficients were computed for ATEN, HYD, LOS and VAL.

Limit of detection and limit of quantification

A calibration curve was prepared using concentrations in the range of 0.16 - 0.24mg/ml for ATEN and range of 0.06 - 0.14mg/ml for HYD, LOS and VAL. The standard deviations of y-intercepts of regression lines were determined and kept in the following equation for the determination of detection limit and quantitation limit. Detection limit = 3.3σ /s; quantitation limit = 10σ /s; where in σ is standard deviation of y-intercepts of regression lines and s is the

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slope of the calibration curve. Detection limit and quantitation limit can also be estimated using signal to noise and relative standard deviation method.

Precision and Accuracy of method (Recovery Studies)

Atenolol: The content of twenty tablets of BP-NOL tablets were taken and weighed. Powder equivalent to 50mg of ATEN was accurately weighed and transferred to 50ml volumetric flask and 30ml of diluent water - acetonitrile (1:1) was added to the same and flask was sonicated for 15.0min. The flask was shaken and the volume was diluted up to the mark with the same mixture. The above solution was filtered using Whatman filter paper (No. 1). Appropriate volume of the aliquot of Atenolol stock solution was taken in different 25ml volumetric flasks and the final volume was made up to the mark with diluent water : acetonitrile (1:1) to obtain 220, 200 and 180µg/ml of ATEN. The drug content per tablets of the above brand of ATEN was calculated from the absorbance values obtained (taking average of three determinations).

Hydrochlorothiazide, Losartan and Valsartan: The content of twenty tablets of COSART-H tablets and VALENT capsules were taken separately and weighed. Powder equivalent to 50mg of HYD, LOS and VAL were accurately weighed and transferred to 100ml volumetric flasks separately and 50ml of diluent water - acetonitrile (1:1) was added to the same and flasks were sonicated for 15.0min. The flasks were shaken and the volumes were diluted up to the mark with the same mixture. The above solution was filtered using Whatman filter paper (No. 1). Appropriate volume of the aliquot of HYD, LOS and VAL stock solution were taken in different 25ml volumetric flasks and the final volume were made up to the mark with diluent water : acetonitrile (1:1) to obtain 120, 100 and 80µg/ml of HYD, LOS and VAL . The drug content per tablet and capsule of the above brand of HYD, LOS and VAL was calculated from the absorbance values obtained (taking average of three determinations).

Results and discussion

Optimization of mobile phase or chromatographic condition was performed based on resolution, tailing factor, symmetric factor and peak areas obtained for ATEN, HYD, LOS and VAL are shown in Table No.1. The precision of an analytical method is expressed as RSD of a series of measurements. It was ascertained by replicate estimation of drug by the proposed method. The values of RSD was within the prescribed limit of 2 %, showing high precision of method, as shown in Table No.2. The plot of peak area of standard solution versus concentration was found to be in the range of 160-240µg/ml for ATEN and 60-140µg/ml for HYD, LOS and VAL respectively and correlation coefficient (r) was 1.000 for ATEN. Similarly the with correlation coefficient (r) was 1.000 for HYD, LOS and VAL respectively**.** The calibration curve for ATEN, HYD, LOS and VAL was obtained by plotting the peak areas of ATEN, HYD, LOS and VAL versus concentrations over a range of 160, 180, 200, 220, 240µg/ml for ATEN and 60, 80, 100, 120, 140µg/ml for HYD, LOS and VAL respectively. ATEN was found to be linear with regression (R^2) = 0.999. Similarly the calibration curves for HYD, LOS and VAL were found to be linear with regression (R^2) = 1.000, are shown in Table No.3. The mobile phase potassium dihydrogen phosphate (10mmol) adjusted to pH 3.0 using diluted ortho phosphoric acid and acetonitrile in the composition (50:50) was found to be satisfactory. These mobile phase composition gave symmetric and well-resolved peaks for ATEN, HYD, LOS and VAL. The resolution between the ATEN and HYD was found 6.29 and resolution between HYD and LOS was found 12.3 and resolution between LOS and VAL was found 8.2, which indicate good separation of these four compounds. The retention time for ATEN, HYD, LOS and VAL was about 1.99min, 2.90min, 5.92min and 9.42min respectively (figure.1). The symmetric factor for ATEN, HYD, LOS and VAL were 0.82, 0.83, 0.81 and 0.80 respectively. UV spectra of ATEN, HYD, LOS and VAL showed that these drugs absorbs appreciably at 210nm. Hence 210nm was selected as the detection wavelength in the liquid chromatography.

The detection limit of ATEN was found 0.2µg/ml. Similarly for HYD was found 0.05µg/ml, LOS was found 0.02µg/ml and VAL was found 0.02µg/ml. The quantification limit for ATEN was found 0.5µg/ml. Similarly HYD was found 0.1µg/ml, LOS was found, 0.1µg/ml and VAL was found 0.05µg/ml respectively, which suggest that these compounds can be estimated accurately. The system suitability parameters were summarized in Table No.4. The average percentage recovery of ATEN was 98.1% to 98.5%, HYD was 99.7% to 100.08%, LOS was 99.4% to 99.84% and VAL was 98.8% to 99.3 % respectively. The recovery of ATEN was found in tablets in the range of 98.1 mg to 98.5 mg. The recovery of HYD was found in the range of 12.46mg to 12.51mg, LOS was found in the

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range of 49.72mg to 49.92mg and VAL was found in the range of 39.52mg to 39.72mg. The chromatographic method was applied to the determination of ATEN, HYD, LOS and VAL in their pharmaceutical dosage form. The results for ATEN, HYD, LOS and VAL were comparable with their corresponding labeled amounts are shown in Table No.5. Ruggedness test was carried out by repeating the procedure under different condition, i.e., on different days, different reagent (make), different column and different HPLC system.

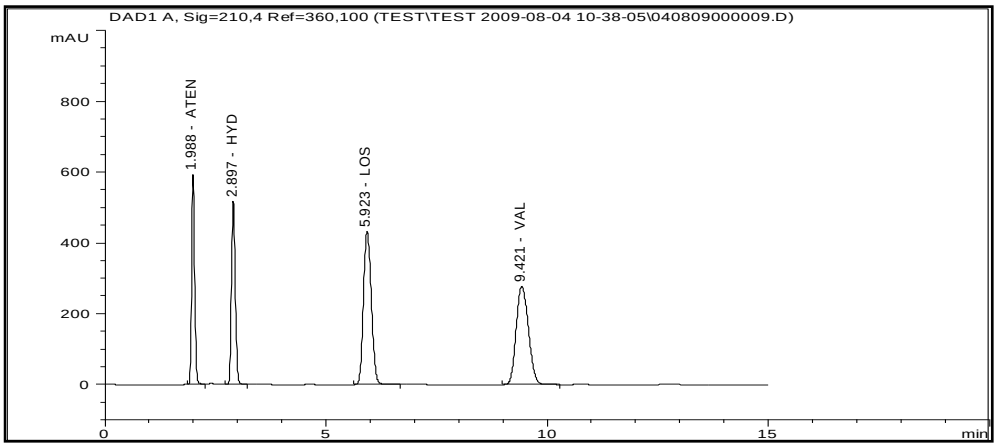

Fig. 1: Typical Chromatogram of ATEN, HYD, LOS and VAL.
Chromatogram showing well resolved peaks of ATEN, HYD, LOS and VAL.
ATEN is Atenolol, HYD is Hydrochlorothiazide, LOS is Losartan and VAL is Valsartan.

Table 1: Optimization of experimental conditions

Parameters	Optimized condition
Chromatograph	Agilent HPLC
Column	Nucleodur100 C-18 , 250 x 4.6 mm , 5µm
Mobile phase	10 mmol KH_2PO_4 pH-3.0 : Acetonitrile (50:50 v/v)
Flow rate	1.0 ml per min.
Detection	UV at 210nm
Injection volume	10µl
Temperature	Ambient
Run Time	15.0 min.
Retention time – ATEN	1.99 min.
Retention time – HYD	2.90 min.
Retention time – LOS	5.92 min.
Retention time – VAL	9.42 min.

* Water HPLC grade filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated.
ATEN is Atenolol, HYD is Hydrochlorothiazide, LOS is Losartan and VAL is Valsartan.

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Table 2: Precision

Inj.	ATEN		HYD		LOS		VAL	
No.	RT(min)	Area	RT(min)	Area	RT(min)	Area	RT(min)	Area
1.	1.99	2819.2	2.90	3098.9	5.92	5348.2	9.42	5357.6
2.	1.99	2825.3	2.90	3103.0	5.93	5360.6	9.44	5366.8
3.	1.99	2834.6	2.90	3115.8	5.93	5377.1	9.44	5385.9
4.	1.99	2843.3	2.90	3125.1	5.93	5399.3	9.44	5402.1
5.	1.99	2856.5	2.90	3138.3	5.93	5418.7	9.44	5430.1
6.	1.99	2864.1	2.90	3145.6	5.92	5430.6	9.43	5442.2
Mean	1.99	2840.5	2.90	3121.1	5.93	5389.1	9.44	5397.5
SD	0.00	17.55	0.00	18.77	0.01	32.65	0.01	33.91
%RSD	0.00	0.62	0.00	0.60	0.09	0.61	0.09	0.63

ATEN is Atenolol, HYD is Hydrochlorothiazide, LOS is Losartan and VAL is Valsartan.
SD is Standard deviation, RSD is Relative standard deviation, Inj. is Injection and RT is Retention time.

Table. 3: Regression analysis of the calibration curves for the proposed method

Parameters	ATEN	HYD	LOS	VAL
Linearity range (µg/ml)	160 – 240	60 – 140	60 – 140	60 – 140
Slope	13357.5	30845.5	50308.5	49428.5
Intercept	1837.38	1245.4	1980.2	1949.7
Standard deviation of slope	1418.9	1756.6	2840.0	2792.3
Regression (R^2)	0.9994	0.9999	0.9998	0.9998
Correlation coefficient (r)	1.000	1.000	1.000	1.000

ATEN is Atenolol, HYD is Hydrochlorothiazide, LOS is Losartan and VAL is Valsartan.

Table 4: System suitability parameter

Parameters	ATEN	HYD	LOS	VAL
Linearity range (µg/ml)	160 – 240	60 – 140	60 – 140	60 – 140
Theoretical plates (meter)	15625	20546	20495	20843
Resolution	---	6.29	12.3	8.20
Tailing factor	1.17	1.15	1.14	1.15
Symmetry factor	0.82	0.83	0.81	0.80
Detection limit (µg/ml)	0.2	0.05	0.02	0.02
Quantification limit (µg/ml)	0.5	0.1	0.1	0.05

ATEN is Atenolol, HYD is Hydrochlorothiazide, LOS is Losartan and VAL is Valsartan.

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Table 5: Assay of combined dosage form and recovery studies

Drugs	Level	Average assay recovery (%)	Amount obtained mg per tablets	Labeled amount Mg per tablets
ATEN	I	98.1	98.1	100.0
	II	98.5	98.5	
	III	98.5	98.5	
HYD	I	99.74	12.47	12.5
	II	99.70	12.46	
	III	100.08	12.51	
LOS	I	99.50	49.75	50.0
	II	99.44	49.72	
	III	99.84	49.92	
VAL	I	99.30	39.72	40.0
	II	98.80	39.52	
	III	98.80	39.52	

ATEN is Atenolol, HYD is Hydrochlorothiazide, LOS is Losartan and VAL is Valsartan.

Table 6: Ruggedness - assay of combined dosage form and recovery studies

Drugs	Level	Average assay recovery (%)	Amount obtained mg per tablets	Labeled amount mg per tablets
ATEN	I	99.30	99.30	100.0
	II	99.40	99.40	
	III	99.60	99.60	
HYD	I	99.80	12.47	12.5
	II	99.70	12.46	
	III	99.75	12.47	
LOS	I	99.80	49.90	50.0
	II	99.70	49.85	
	III	99.60	49.80	
VAL	I	100.10	40.04	40.0
	II	100.00	40.00	
	III	100.04	40.01	

ATEN is Atenolol, HYD is Hydrochlorothiazide, LOS is Losartan and VAL is Valsartan.

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In ruggedness test recovery studies of ATEN was found in the range of 99.30% to 99.60%, HYD was found in the range of 99.70% to 99.80%, LOS was found in the range of 99.60% to 99.80% and VAL was found in the range of 100.0% to 100.10% respectively. The recovery of ATEN was found in tablets in the range of 99.30mg to 99.60mg. The recovery of HYD was found in the range of 12.46mg to 12.47mg, LOS was found in the range of 49.80mg to 49.90mg and VAL was found in the range of 40.00mg to 40.04mg. which are shown in Table No.6.

The reversed-phase HPLC method was developed to provide a specific procedure suitable for the rapid quality control analysis ATEN, HYD, LOS and VAL combination in mixture using simple mobile phase potassium dihydrogen phosphate and acetonitrile compared to the reported method. Specificity of the methods was determined by the complete separation of ATEN, HYD, LOS and VAL with other parameters like retention time, tailing factor and capacity factor. Rapidity and capability of qualifying very low concentration of respective drugs, made them useful for variety of analyses, including pure drug analysis, assay of formulations and stability studies analysis. The purposed method did not utilize any extraction step for recovering the drug from the formulation excipient matrixes and their by decreased the degree of error, time in estimation of the drugs and the overall cost of the analysis. The solvent system used was simple mobile phase compared to the reported method. The method gives good resolution between these four compounds with a short analysis time (less than 15.0 minute). The method was validated and found to be simple, sensitive, accurate, precise and economical. Percentage of assay 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 ATEN, HYD, LOS and VAL in their pharmaceutical dosage form.

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