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Development And Validation Of RP-HPLC Method For Analysis Of Aliskiren Hemifumarate And Valsartan In Their Combination Tablet Dosage Form

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Abstract: Aliskiren Hemifumarate and Valsartan belong to a group of Anti-hypertensive drugs. A Simple, Rapid, Specific and economic Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed for assaying both the drugs in combinational dosage form. Method involves elution of Aliskiren Hemifumarate and Valsartan in Hyper ODS2, Column C_{18}, 250 x 4.6 mm (5 µm) using mobile phase composition of Acetonitrile: 0.05M Potassium dihydrogen phosphate buffer, pH 3.5 adjusted with O-Phosphoric acid (45:55, v/v), at flow rate 1ml/min and analytes were monitored at 224nm.Method has been validated according to ICH (International Conference on Harmonization) Guideline. Method shows good linearity over the range of 10-50µg/ml for both the drugs. All the validation parameters were within the range. The developed method was successfully applied to estimate the amount of Aliskiren Hemifumarate and Valsartan in Tablet and synthetic mixture.
Keywords: Aliskiren Hemifumarate, Valsartan, RP-HPLC, Validation.

Introduction:

Aliskiren Hemifumarate (ALK) (2s,4s,5s,7s)-5-amino-n-(2-carbamoyl-2,2-dimethylethyl)-4-hydroxy-7-{[4-methoxy-3-(3-methoxypropoxy)phenyl]methyl}-8-methyl-2-(propan-2-yl) nonanamide[2] (Fig. 1) is an orally active renin inhibitor that is use in hypertension and heart failure[2]. It is not official in any Pharmacopoeia. Literature survey reveals Spectrophotometric Methods[5], RP-HPLC[6] for determination of ALK with other drugs. Valsartan (VAL) (2s)-3-methyl-2-[n-({4-[2-(2h-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl) pentanamido] butanoic acid [1] (Fig. 1) is Angiotensin II receptor antagonist; it is used in the management of hypertension, to reduce cardiovascular mortality in patients with left ventricular dysfunction following myocardial infarction, and in the management of heart failure.It is official in British Pharmacopoeia (BP) and United States Pharmacopoeia (USP), BP[1] describe High Performance Liquid Chromatography (HPLC) and USP[3] also describe HPLC method. Literature survey also reveals Spectrophotometric Methods[7], RP-HPLC[8], HPTLC[9], UPLC[10], LCMS[11] for determination of VAL with other drugs.

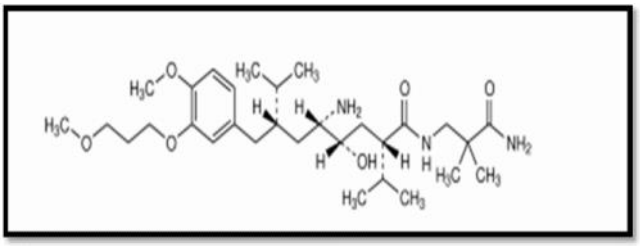

Fig. 1: Structure of Aliskiren Hemifumarate

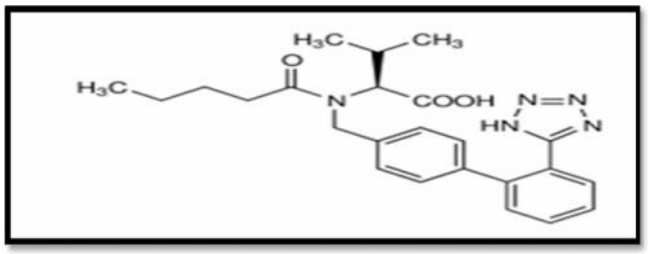

Fig. 2: Structure of Valsartan

The combined dosage form of ALK and VAL is also available in the market for Hypertension. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for simultaneous estimation of ALK and VAL in their combined dosage form. Literature survey does not reveal any simple Spectrophotometric or other method for simultaneous estimation of ALK and VAL in combined dosage form. Hence aim of work is to develop Simple, Rapid, Specific and economic Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method for routine analysis of ALK and VAL in their combined dosage form.

In present study Simple, Rapid, Specific and economic RP-HPLC method for estimation of Aliskiren Hemifumarate and Valsartan in their combined pharmaceutical dosage form is reported.

Experimental:

1.1 Material And Method:

1.2 Chemicals And Reagents:

ALK and VAL were kindly given as a gratis sample by Relax Pharmaceuticals, Makarpura, Baroda, Gujarat, India. The market formulation VALTURNA (ALK 150 mg and VAL 160 mg) was procured from Indian market which is manufactured by Novartis pharmaceutical, Bombay, India. Acetonitrile (HPLC Grade) and Water(HPLC Grade) were obtained from RFCL limited, New Delhi. All other reagents Potassium dihydrogen orthophosphate and O-phosphoric acid of analytical grade were purchased from SD Fines chemicals, Bombay.

1.3 RP-HPLC Instrumentation And Conditions:

The HPLC(Analytical technologies limited) system consisted ofP2230 plus HPLC pump,Rhenodyne valve with 20µl fixed loop, UV 2230 plus detector, Analchrom 2006 Software, The chromatographic separation achieved on a Hyperchrom ODS-BP Column, (5µm, 200mm x 4.6mm i.d.) using a mobile phase consisted of Acetonitrile: 0.05M Potassium dihydrogen phosphate buffer, pH 3.5 adjusted with O-Phosphoric acid (45:55, v/v), at flow rate 1ml/min and analytes were monitored at 224nm.

1.4 Preparation Of Stock And Standard Solution:

1.4.1 Stock solution of ALK:

A 100mg of standard ALK accurately was weighed and transferred to a 100 ml volumetric flask and dissolved in 50 ml mobile phase. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 1000 µg/ml ALK. From this solution 2.5 ml was transfer to 25 ml volumetric flask. The volume was adjusted to the mark with the mobile phase to give a solution containing 100µg/ml ALK.

1.4.2 Stock solution of VAL:

A 100 mg of standard VAL was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 50 ml mobile phase. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 1000 µg/ml VAL. From this solution 2.5 ml was transfer to 25 ml volumetric flask. The volume was adjusted to the mark with the mobile phase to give a solution containing 100µg/ml VAL.

1.4.3 Calibration Standard solutions of ALK and VAL:

Appropriate volume of aliquot from ALK and VAL stock solution was transferred to same volumetric flask of 10 ml capacity. The volume was adjusted to the mark with mobile phase to give a solution containing 10, 20, 30, 40 and 50 µg/ml ALK and 10, 20, 30, 40 and 50µg/ml VAL.

1.5 Preparation Of Sample Solution For Tablet Assay:

Twenty tablets were weighed and finely powdered. The powder equivalent to 15 mg ALK and 16 mg VAL was accurately weighed and transferred to volumetric flask of 100 ml capacity. 50 ml of mobile phase was transferred to volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase. The above solution was filtered through whatmann filter paper (0.45µ). 2.5 ml of aliquot was taken and transferred to volumetric flask of 25 ml capacity and volume was made up to the mark with the mobile phase to give a solution containing 30 µg/ml ALK and 16 µg/ml VAL which were in the range of linearity previously described. This sample solution was used for the estimation of ALK and VAL.

2. Result And Discussion:

2.1 HPLC Method Development And Optimization:

Hyperchrom ODS-BP 5µm, 200mm x 4.6mm i.d. Column (Analytical technologies limited) maintained at ambient temperature was used for the separation and the method validated for estimation of ALK and VAL in their combined tablet dosage form. The composition, pH, Flow rate of mobile phase changed to optimize the separation condition. A mobile phase consisting of ACN and 0.05M Potassium dihydrogen phosphate buffer, pH 3.5 adjusted with O-Phosphoric acid (45:55, v/v) with gradient elution was selected for use for further studies after several preliminary investigatory chromatographic runs (**Table-1).** Under described conditions, all peaks were well defined and free from tailing.

2.2 Validation Of HPLC Method:

2.2.1 Linearity:

Linearity was established by least square linear regression analysis of the calibration curve. The constructed calibration curves were linear over the range of 10-50 µg/ml for both the drugs. Peak area of ALK and VAL were plotted versus their respective concentrations and linear regression analysis was performed on the resultant curves. Typically, the regression equations were: y =14.1398 x + 128.4802 (R^2 = 0.9985), y = 43.7753 x + 710.3610 (R^2 = 0.9998) for ALK and VAL respectively (**Table-2).**

2.2.2 LOD and LOQ:

LOD and LOQ were performed on samples containing concentrations of analytes, based on calibration method. Standard solution of ALK and VAL were injected in six replicate (**Table 2).** Average peak area of six analyte was plotted against concentration. LOD and LOQ were calculated using following equation,

LOD=(3.3 x σ) / S

LOQ = (10 x σ) / S

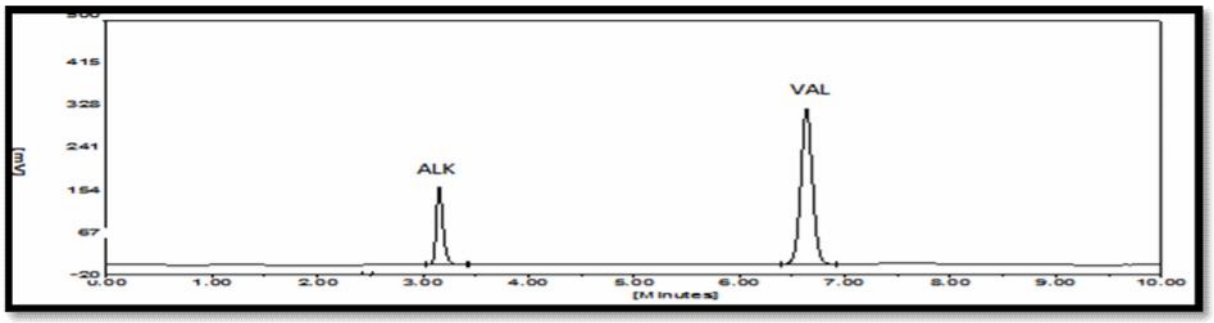

Fig 2: Chromatogram of mixed standard solution containing 40 µg/ml ALK and 40µg/ml VAL using Acetonitrile: 0.05M Potassium dihydrogen phosphate buffer pH-3.5 adjusted with Orthophosphoric Acid (45:55%v/v) mobile phase at 224nm

Table 1: Various Mobile phases tried for optimization

Mobile Phase	Proportion (v/v)	Detection Wavelength (nm)	Flow rate (ml / min)
Acetonitrile : Water	50:50	224	1
Acetonitrile : Buffer, pH 7	50:50	224	1
Acetonitrile : Buffer, pH 5	50:50	224	1
Acetonitrile : Buffer, pH 4	50:50	224	1
Acetonitrile : Buffer, pH 3	50:50	224	1
Acetonitrile : Buffer, pH 3.5	50:50	224	1
Acetonitrile : Buffer, pH 3.5 (proposed mobile phase)	45:55	224	1

Table 2: Statistical data for ALK and VAL by RP- HPLC method

Parameter	ALK	VAL
Linear Range(µg/ml)	10 - 50	10 – 50
Slope	14.18	43.77
Intercept	125.57	710.36
Standard deviation of slope	0.34	0.37
Standard deviation of intercept	11.31	12.44
Limit of Detection (µg/ml)	2.63	0.93
Limit of Quantitation (µg/ml)	7.97	2.84

2.2.3 System suitability:

System suitability parameters can be defined as a test to ensure that the method can generate results of acceptable accuracy and precision. System suitability parameters like Retention time, Resolution, theoretical plates, tailing factor were calculated and compared with standard values to ascertain whether the proposed RP-HPLC method for the estimation of ALK and VAL in pharmaceutical dosage form was validated or not. Results are shown in Table-3.

2.2.4 Accuracy:

A known amount of each standard powder (80%, 100%, and 120%) was added to the synthetic mixture of excipients and subsequently diluted to yield a starting concentration of 12µg/ml, 15µg/ml and 18µg/ml for ALK and 12.8µg/ml, 16µg/ml and 19.2µg/ml for VAL. The observed % recovery was ranging from 99.12-99.79% for ALK and 99.32-99.92% for VAL (Table-4).

2.2.5 Precision:

The Interday intraday variability data are summarised in Table-4. They were assessed by using standard solutions to produce solutions of three different concentrations of each drug. Intraday precision investigated by injecting three replicate sample of each of sample of three different concentrations. Intraday precision were assessed by injecting same three samples over three consecutive days (Table-4).

2.2.6 Repeatability:

Standard mixture solutions of ALK (10, 20, 30, 40 & 50 µg/ml) and VAL (10, 20, 30, 40 & 50 µg/ml) were prepared and chromatograms were recorded. Area was measured of the same concentration solution six times and C.V. was calculated (Table-4).

Table 3: system suitability parameters:

Parameter	ALK	VAL	Range	Inference
Retention time(Rt)(minutes)±S.D.	3.146±0.041	6.5±0.258	-	-
Peak width (minutes)±S.D.	0.116±0.005	0.204±0.011	-	-
Resolution(Rs)	----------20.96----------		>2	Criteria met
Tailing factor±S.D.	1.454±0.043	1.068±0.060	<2	Criteria met
Theoretical Plates(Plates/Meter)	11,025	16,242	Above 2000	Criteria met

Table 4: Summary of Validation Parameters of RP-HPLC

Parameters	ALK	VAL
Recovery %	99.12-99.79	99.32-99.92
Repeatability (RSD, n=6)	0.0038	0.0025
Precision(CV)		
Intra-day (n=3)	0.53-0.82	0.45-0.81
Inter-day (n=3)	0.51-1.03	0.34-0.57
Specificity	Specific	specific
Solvent suitability	Solvent suitable for 48 hrs	Solvent suitable for 48 hrs

Table 5: Assay Results of Marketed Formulation

Formulation	Actual concentration µg/ml		Amount obtained µg/ml		% ALK±S.D.	% VAL±S.D.
	ALK	VAL	ALK	VAL		
Tablet	15	16	14.89	15.87	99.31±0.44	99.24±0.47

2.3 Assay:

Validated method was applied for the determination of ALK and VAL in commercially available VALTURNA tablets. The result of assay undertaken yielded 99.31% and 99.24% of label claim for ALK and VAL respectively (Table-5).

Conclusion:

A Simple, Rapid, Specific and economic Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for routine analysis of ALK and VAL in API and combinational dosage forms. The proposed method has ability to separate these drugs from excipients found in tablet dosage form.

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