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RESEARCH ARTICLE

DEVELOPMENT & VALIDATION OF HPLC METHOD FOR ANALYSIS OF SOME ANTIHYPERTENSIVE AGENTS IN THEIR PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, fast, specific and accurate reverse-phase high performance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for simultaneous estimation of Aliskiren (ALN) and Felodipine (FLD) from their dosage forms. Agilent 1200 infinity LC equipped with UV-Visible with EZChrom Elite compact version 3.3.2 SP2 software was used. Chromatographic separation was achieved isocratically on a Hypersil BDS C8 column (150×4.6 mm, 5 µ particle size) using a mobile phase, Water (adjusted to pH 3.0 with 10%v/v ortho-phosphoric acid) , Acetonitrile and Methanol in the ratio of 20:30:50 v/v/v and UV detection was carried out at 254 nm for both the drugs ALN and FLD. The retention time for ALS and FLD was found to be 4.26 and 2.96 min respectively, and recoveries were found between 98 and 102%. The method was found linear over the range of 150-450 µg mL^{-1} for ALS and 5-15 µg mL^{-1} for FLD. The method has been successfully applied to assess the assay of solid dosage formulations.

Key words: Aliskiren hemifumarate; Felodipine; RP-HPLC Method;

INTRODUCTION

Hypertension or **high blood pressure** is a chronic medical condition in which the blood pressure in the arteries is elevated. It is classified as either primary (essential) or secondary. About 90-95 % of cases are termed "primary hypertension", which refers to high blood pressure for which no medical cause can be found the remaining 5-10 % of cases (Secondary hypertension) are caused by another conditions that affect the kidneys, arteries, heart, or endocrine system. Blood pressure is classified based on two types of measurements, the systolic and diastolic blood pressures expressed as a ratio such as '120 over 80' (120/80) mmHg. Systolic blood pressure is the blood pressure in vessels during a heartbeat. Diastolic blood pressure is the pressure between heartbeats.

Aliskiren is a (ALS),(2S, 4S, 5S, 7S)- N-(2-carbamoyl-2-methylpropyl)-5-amino-4- hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide hemifumarate (Figure-1).The first oral direct rennin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Aliskiren blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotensin II[1].

it is a highly potent and selective inhibitor of human renin in vitro, and in vivo; once-daily oral doses of Aliskiren inhibit renin and lower blood pressure in sodium-depleted marmosets and hypertensive human patients. Aliskiren represents the first in a novel class of renin inhibitors with the potential for treatment of hypertension and related cardiovascular diseases. Aliskiren administered both orally or intravenously. Aliskiren is also available as combination therapy with Amlodipine and Hydrochlorothiazide.

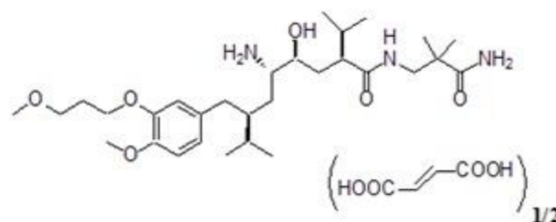

Figure 1; Chemical structure of Aliskiren

Felodipine is a long-acting 1,4-dihydropyridine calcium channel blocker . Felodipine is a dihydropyridine derivative that is chemically described as ethyl methyl (RS) 4-(2, 3-dichlorophenyl) - 1, 4-dihydro-2, 6-dimethylpyridine-3, 5-dicarboxylate (Figure-2). It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, Felodipine prevents calcium-dependent myocyte contraction and vasoconstriction[2],[3]. Felodipine is the most potent CCB in use and is unique in that it exhibits fluorescent activity. In addition to binding to L-type calcium channels, Felodipine binds to a number of calcium-binding proteins, exhibits competitive antagonism of the mineralcorticoid receptor, inhibits the activity of calmodulin-dependent cyclic nucleotide phosphodiesterase, and blocks calcium influx through voltage-gated T-type calcium channels. Felodipine is used to treat mild to moderate essential hypertension[4],[5],[6].

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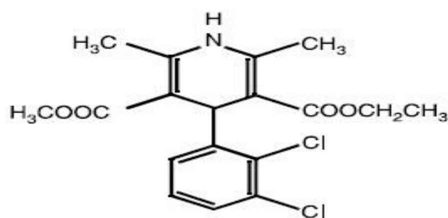

Figure 2: Chemical structure of Felodipine

MATERIAL AND METHODS:

Experimental Chromatographic conditions

The analysis of the drug was carried out on a Agilent 1200 infinity LC system equipped with EZChrom Elite compact version 3.3.2 SP2 software using Hypersil BDS C8 column (150×4.6 mm, 5 µ particle size) at ambient temperature. A degassed mixture of Water (adjusted to pH 3.0 with 10% ortho-phosphoric acid) , Acetonitrile and Methanol in the ratio of 20:30:50 v/v/v was used as mobile phase and detection was carried out at 254 nm.

Chemicals and Solvents

The Aliskiren (ALS) and Felodipine (FLD) Working standards were kindly gifted by Morepen Laboratories Ltd, New Delhi and AUM Research Laboratories, Ahmedabad-India. HPLC grade methanol and Acetonitrile were purchased from FINAR Limited. Ortho-phosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd.

Preparation of mobile phase and diluent

Mobile phase:

500 ml of Milli-Q water was adjusted to pH 3.0 using 10%v/v ortho-phosphoric acid and filtered through 0.45filter under vacuum. 200 ml of water (adjusted to pH 3.0 with 10%v/v ortho-phosphoric acid) was mixed with 300 ml of Acetonitrile and 500 ml of Methanol and the resulting mixture was degassed in an ultrasonic water bath for 15 minutes.

Diluent:

A mixture of Water and Methanol in ration of 50:50v/v used as diluent.

Procedure

A mixture of water (adjusted to pH 3.0 with 1% ortho-phosphoric acid) and Acetonitrile and Methanol in the ratio of 20:30:50 v/v was found to be the most suitable mobile phase for separation of Aliskiren and Felodipine. The solvent mixture was sonicated before use. It was pumped through the column at a flow rate of 1.0 ml/min. The column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at wavelength 254 nm. The injection volume was set 10µl and sample run time was set at 6 min. Under these optimized chromatographic conditions the retention time obtained for the drugs ALS and FLD were 4.26 and 2.95 min respectively. System suitability data are summarized in Table 1. A typical chromatogram showing the separation of the drugs is given in Figure-3.

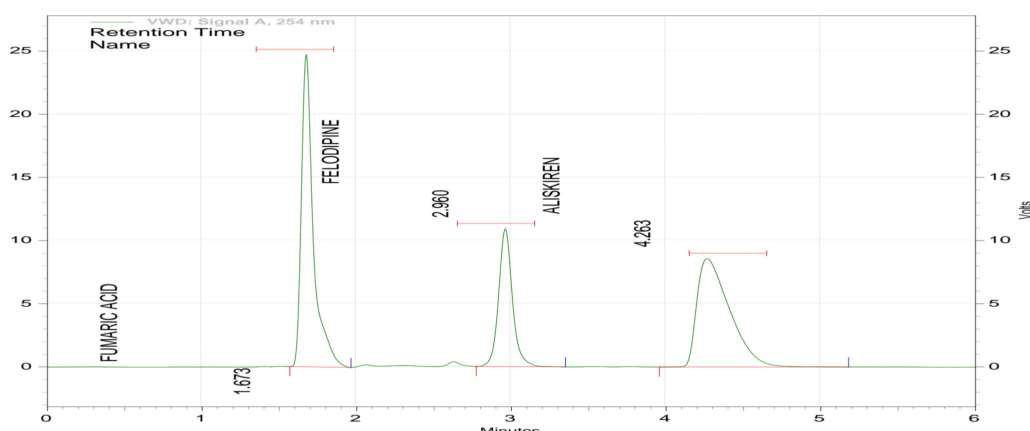

Figure 3: Chromatogram depicting for Fumaric acid, Felodipine and Aliskiren peak

Linearity study:

Stock solution preparation:

About 150 mg of ALS and 5 mg of FLD was weighed accurately, transferred into a individual 50 ml volumetric flask and dissolved in 5 ml of methanol added and sonicated to dissolve for 5 minutes. Make up the volume with diluent (i.e. mixture of Water and Methanol in ratio of 50:50 v/v).

Mix Standard preparation:

Mix standard preparation of both the drugs were prepared by dilution of the standard stock preparation with diluent to obtain the concentration range of 300 µg mL^{-1} and 10 µg mL^{-1} for ALS and FLD respectively.

Linearity solution preparation:

Linearity solution was prepared by dilution of standard stock solution with diluent to obtained linearity concentration rage of 150 - 450 µg mL^{-1} and 5-15 µg mL^{-1} for ALS and FLD respectively.10 µl of Five injections of mixed standard solution preparation and single injection of linearity solution was injected into the column at a flow rate of 1.0 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each preparation was computed. The Linearity graph constructed by plotting concentration of the drug against

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peak area was found to be linear in the concentration range of 150-450 µg mL^{-1} for ALS and 5-15 µg mL^{-1} for FLD. The regression equations of this curves was computed. The relevant data are furnished in Table 1 & 2 and figure 4 & 5.

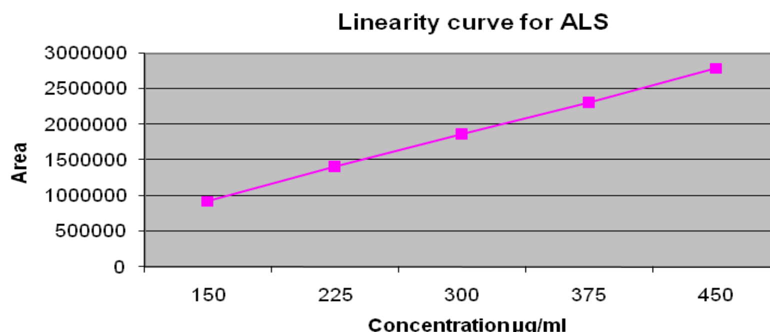

Figure 4: Linearity Graph for Aliskiren Peak.

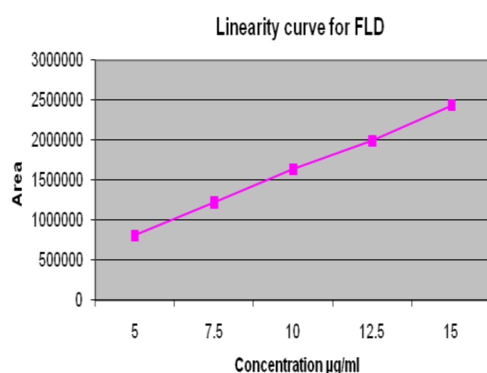

Figure 5: Linearity Graph for Felodipine Peak.

Specificity of the proposed method:

Identification solution reparation:

Identification solution for ALS:

Identification solution was prepared by dilution of the standard stock preparation with diluent to obtain the concentration at about 300 µg mL^{-1} for ALS.

Identification solution for FLD:

Identification solution was prepared by dilution of the standard stock preparation with diluent to obtain the concentration at about 10 µg mL^{-1} for FLD.

Identification of Fumaric acid peak:

Identification solution was prepared by using Fumaric acid reagent and diluent. The solution of about 30 µg mL^{-1} of Fumaric acid solution was used and injected as per method.

Placebo preparation:

Identical mix placebo was prepared for ALS and FLD formulations and injected for placebo interference study.

Above prepared solution were analyzed by using proposed method. Obtained chromatogram were evaluated for Interference of the Fumaric acid and placebo. The relevant data are furnished in Table 1.

Accuracy of the proposed method:

The accuracy of the HPLC method was assessed by analyzing solutions of ALS and FLD at 50, 100 and 150% concentration level.by the proposed method. The accuracy of the method was established based on the summary given in Table 1

Table 1: Chromatographic Data

	ALS peak	**FLD peak**
Theoretical plate	>2000	>5000
Asymmetry	1.15	1.80
Resolution from previous peak	> 4.5	>9.0
Linearity range (µg/ml)	150-450 µg mL^{-1}	5-15 µg mL^{-1}
Correlation coefficient	0.999	0.999
Limit of Quantification	3 µg mL^{-1}	0.1 µg mL^{-1}
%RSD (n=5)	0.34	1.02
Recovery	98-102%	98-102%
Specificity	Specific	Specific

Table 2: Linearity Study for ALS and FLD

ALS peak		FLD peak	
Concentration in µg mL^{-1}	Peak Area	Concentration in µg mL^{-1}	Peak Area
150	925019	5	810028
225	1411699	7.5	1219612
300	1868121	10	1633368
375	2310896	12.5	1988990
450	2787431	15	2427710

DISCUSSION

In the proposed method, the retention time of ALS and FLD was found to be 4.26 and 2.95 minute. Quantification was linear in the concentration range of 150- 450µg/ml for ALS and 5-15µg/ml for FLD. The regression equation of the linearity plot of concentration of ALS over its peak area was found to be y = 46240x + 47342(r2=0.999) for ALS and y = 40047x + 41451 (r2=0.999) for FLD, where X is the concentration of ALS and FLD (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 2000 for ALS and 3000 for FLD, which indicates efficient performance of the column. The limit of quantification for ALS were found to be 3.0µg/ml and for FLD were found to be 0.1 µg/ml, which indicate the sensitivity of the method. The recovery study indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in formulations did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION

The developed HPLC method was validated and the statistical validation was performed with the simplicity, and ease of operation ensures that the validated method can successfully used for routine analysis of Aliskiren hemifumarate and Felodipine in bulk and formulation. It is of practical utility because all the molecules are available in individual formulations or will be available in its future combinations. The application of this method in routine analysis can be justified since easy sample preparation steps are involved with simple reagents and solvents were used experimentally. The method can be employed in quality control of pharmaceuticals containing Aliskiren and Felodipine to reduce analytical time.

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