Original Research Article

VALIDATION OF HPLC AND UV VISIBLE METHODS FOR FEW SELECTED BLOOD PRESSURE LOWERING DRUGS & THEIR FORMULATIONS

ABSTRACT

A simple, precise and accurate RP-HPLC method has been developed and subsequently validated for simultaneous estimation of Aliskiren Hemifumarate and Nicardipine Besylate from their combination dosage form. Aliskiren and Nicardipine are widely used antihypertensive drugs at present but their analytical methods are very costly & very complex to simplify the methods with increasing sensitivity new methods were developed which are simple, precise, eco-friendly, less time consuming, rapid & fast & economically chief. First standard curve was plotted then the method is validated by using recovery studies, linearity, correctness & reproducibility, robustness, ruggedness, detection limit, quantification limits, stability studies etc. The validated technique has been with success used for stress testing analysis of Aliskiren & Nicardipine. The stress testing studies revealed that the tactic was with success utilized to resolve the degraded product from the sample. From the peak purity profile it had been demonstrated that there was no interference of degradation product & the purity of angle were found to be but the purity of threshold. This work was undertaken with an aim of developing HPLC and Specrophotometric techniques for analysis of Aliskiren and Nicardipine. Number of trials was taken for selection of column & M. Phase'. The proposed method was validated as per the ICH and USP guidelines.

Keywords: Aliskiren, Nicardipine, RP-HPLC Method; Tablet dosage forms.

INTRODUCTION

Aliskiren is a novel antihypertensive agent & is that the 1st orally active enzyme substance indicated for the treatment of cardiovascular disease. Chemically, Aliskiren is (2(S),4(S),5(S),7(S)-N-(2-carbamoyl-2- methylpropyl)-5-amino-4 hydroxy2,7 diisopropyl -8-[4- methoxy-3-(3-methoxypropoxy)phenyl] octanamide hemifumarate)(Figure 1)[1, 2, 3]. The first oral direct renin 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. Aliskiren represents the first in a novel class of renin inhibitors with the potential for treatment of hypertension and related cardiovascular diseases[4]

Nicardipine is a member of 1, 4-dihydropyridine class of metal antagonist approved for the treatment of heart diseases like cardiovascular disease & angina pectoris. It is a protracted acting metal channel blocker that inhibits the flow of calcium ions into the tube swish muscle & muscular tissue with chemicals Nicardipine is 3-ethyl-5-methyl 2 [(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydropyridine-6-methyl-3,5dicarboxylate(Figure 2).

Through literature survey reveals that there square measure few analytical ways such as RP-HPLC & ultraviolet light ways are rumored for synchronous estimation of Aliskiren & Nicardipine in pharmaceutical dose forms. But therefore way there's no stability indicating technique rumored. Therefore the gift investigation was allotted to develop new easy, precise, rapid, & cost-effective stability indicating RP-HPLC technique for the synchronous estimation of Aliskiren & Nicardipine in pharmaceutical dose kind. Present work emphasizes on the quantitative estimation of Aliskiren and Nicardipine in their combined dosage form (Tablets) by RP-HPLC. The proposed technique was also successfully used to separate the degraded product from the samples.

MATERIALS AND METHODS

Aliskiren & Nicardipine standards were provided from Spectrum Research Laboratory, Hyderabad, & commercial pill dose kind TEKEMLO was purchased from native market. The HPLC grade acetonitrile & water were purchased from Merck & analytical grade potassium dihydrogen phosphate was purchased from RANKEM. Analytical grade triethylamine, orthophosphoric acid, hydrochloric acid, sodium hydroxide, & hydrogen peroxide were purchased from S.D. Fine Chemicals.

Preparation of buffer resolution for mobile phase-A:

Dissolved 1.36 gram metal diatomic number 1 ortho phosphate into 1L of HPLC grade water. Mixed well using a magnetic stirrer bar till fully mixed. The solution was filtered through a zerom nylon membrane filter 45μ & degassed.

Preparation of mobile Phase-B: Acetonitrile (HPLC Grade)

Preparation of diluents: fuel (HPLC Grade)

Intact pills of Aliskiren Hemifumarate & Nicardipine Besylate taken into 500 ml volumetric flask than additional 10 ml of water & sonicated until disintegrated. Added concerning 400 ml of fuel & sonicated for 45 minutes than created up volume with fuel. Added 5 ml of this resolution in to 50 mm volumetric flask & build up volume with fuel & filtered with 0.45 μm nylon filter. A Validated Stability Indicating RP-HPLC technique Development & Validation for synchronous Estimation of Aliskiren Hemifumarate & Nicardipine Besylate in Pharmaceutical dose kind. The present study describes the soundness indicating RP-HPLC technique for synchronous estimation of Aliskiren hemifumarate & Nicardipine besylate in pharmaceutical dose forms.

HPLC Instrument:

The chromatographic separation was carried out by waters 2695 HPLC system separation module (Labtronic) equipped with personal digital assistant detector & autosampler. The Empower 2 package was used for signal observation & process. UV chamber has been used for photolytic degradation & hot air kitchen appliance was utilized for thermal degradation.

Conditions of Chromatography

The chromatographic separation of analytes was carried out exploitation Labtronics RP-HPLC system with C-18 hypersil ODS (150 × four.6 mm, 5 μm) column. The mobile phase consists of phosphate buffer & acetonitrile in the ration of 40 : 60% v/v & hydrogen ion concentration was adjusted to three with phosphoric acid resolution that was wont to separate the analytes & column temperature was maintained at 30°C. The analytes were detected at 237 nm using personal digital assistant detector. The run time was set at 10 min at a flow rate of 1 mL/min.[5,6] Data are provided in table 1.

Standard stock solution preparation

Standard stock solutions of aliskiren & Nicardipine were prepared individually by dissolving 50 mg of Aliskiren & 10 mg of Nicardipine in 10 mL volumetrical flasks with water : acetonitrile (50 : 50% v/v) as dilutant & sonicated for 5 min. From the above resolution transfer 0.3 mL of Aliskiren & 0.1 mL of Nicardipine separately into 10 mL volumetrical flasks & build up the amount with dilutant to induce 150 μg/mL of Aliskiren & 10 μg/mL of Nicardipine standard stock resolution.

Sample solution Preparation

Five pills (TAKEMLO tablets: 150 mg Aliskiren & 10 mg Nicardipine) were weighed & the average weight of every tablet was calculated; then the load such as 5 tablets was transferred into a 250 mL volumetrical flask; 60 mL of dilutant was additional & sonicated for 25 min; and the amount was created up with dilutant & filtered. From the filtered solution 0.5 mL was pipetted out into a 10 mL volumetric flask & created up to 10 mL with dilutant.

Degradation studies by Force[7-10]

Forced degradation studies of the drug formulation were carried out by treating the drug samples under stress evoked conditions like acid & base chemical reaction, oxidation, & photo & thermal degradation & interference of the degraded products was investigated. These studies help to understand the inherent stability characteristic of the active molecules in drug product & the attainable degradation product

Degradation studies by acid[7-10]

To 1 mL stock solution of Aliskiren & Nicardipine, 1 mL of 2N hydrochloric acid was added & refluxed for 30 mins at 60°C. The resultant resolution was diluted to get 150 μg/mL & 10 μg/mL solution & 10 μL solutions were injected into the system & the chromatograms were recorded to assess the soundness of sample.

Degradation studies by acid[7-10]

To 1 mL stock solution of aliskiren & Nicardipine, 1 mL of 2N sodium hydroxide was additional & refluxed for 30 min at 60°C. The sample resolution was ready to get the concentration of 150 μg/mL & 10 μg/mL solution & 10 μL was injected into the system & the chromatograms were recorded to assess the soundness of sample.

Oxidation[7-10]

To 1 mL stock solution of aliskiren & Nicardipine, 1 mL of 20% atomic number 1 peroxide (H2O2) was additional individually. The solutions were kept for 30 min at 60°C. For HPLC study, the sample resolution was ready to get the concentration of 150 μg/mL & 10 μg/mL solution & 10 μL was injected into the system & the chromatograms were recorded to assess the soundness of sample.

Photo stability studies[7-10]

The photochemical stability of the drug was conjointly studied by exposing the 150 μg/mL & 10 μg/mL resolution to light-weight|ultraviolet illumination|UV|actinic radiation|actinic ray} light by keeping the beaker in ultraviolet light chamber for seven days or two hundred watt hours/m2 in photo stability chamber. For HPLC study, the sample resolution was ready to get the concentration of 150 μg/mL & 10 μg/mL solution & 10 μL was injected into the system & the chromatograms were recorded to assess the soundness of sample.

Degradation studies by Dry Heat[7-10]

The standard drug solution was placed in kitchen appliance at 105°C for 6 h to study dry heat degradation. For HPLC study, the sample resolution was ready to get the concentration of 150 μg/mL & 10 μg/mL solution & 10 μL was injected into the system & the chromatograms were recorded to assess the soundness of the sample.

RESULT & DISCUSSION

A series of trials was conducted with completely different columns like Inertsil ODS & agilent XDB C-18 & C-8 columns with different mobile phases to develop a appropriate RP-HPLC technique for estimation of Aliskiren hemifumarate & Nicardipine besylate in pill dose kind, & finally a typical chromatogram was obtained with phosphate buffer & acetonitrile in the ration of 40 : 60% v/v & hydrogen ion concentration was adjusted to three with phosphoric acid at a rate of 1 mL/min. The chromatographic separation was performed on C-8 Inertsil ODS (150 × 4.6 mm, 5 μ) by injecting 10 μL & analytes were detected with PDA detector at 237 nm. The retention time of Aliskiren & Nicardipine was found to be 3.98 & 5.14 min respectively. Forced degradation studies were also carried exploitation the developed technique & the degraded compounds were effectively resolved from the Aliskiren & Nicardipine in pill dose kind.

Method Validation

The validation was performed with above developed RP HPLC technique for synchronous estimation of Aliskiren & Nicardipine according to ICH tips. Various parameters were evaluated such as system quality, precision, accuracy, linearity, robustness, LOD, & LOQ

System Suitability Parameters

System suitability was performed to verify the acceptableness of the resolution & repeatability of the system. System suitability was performed by injecting six replicate injections of the standard resolution (100%) & parameters such as peak space, USP tailing, theoretical plates, retention time, & peak asymmetry were evaluated. The % RSD determined and rumored inside the bounds.

Accuracy

The accuracy of the proposed technique was evaluated by calculating the recovery studies of drug at 3 completely different concentration levels (50%, 100%, & 150%) by standard addition method. A known quantity of Aliskiren & Nicardipine was additional to pre-quantified sample resolution & 3 replicates of every concentration were injected in developed chromatographical conditions. The mean percentage recovery of Aliskiren & Nicardipine was varied between 99.99 & 101.7% indicating that the developed technique was found to be correct.

The precision of Associate in nursing analytical procedure could be outlined because the closeness of agreement between a series of measurements obtained from multiple sampling of an equivalent solid sample underneath the prescribed conditions. The method exactitude & system exactitude studies were allotted by injecting 6 replicates of each standard & take a look at solutions with an equivalent concentration. The % RSD was calculated from the chromatograms & results obtained were inside the bounds of 22 & planned technique was found to be precise.

Precision

The linearity of the technique determined at completely different concentration levels starting from 30 to 55 µg/mL of Aliskiren & from 2 to 15 µg/mL of Nicardipine. All the concentrations were prepared & injected into the system. The linearity curve was created by plotting peak space versus concentration of the analyte. From the results obtained the proposed technique was found to be linear. The regression coefficient was found to be 0.9990 for both Aliskiren & Nicardipine (Figure 3, and 4).

Lim. of Detection & Quantification (LOD & LOQ)

In the present study the LOD & LOQ of Aliskiren & Nicardipine were evaluated supported the standard activity curve technique. Limit of detection is performed to know the bottom concentration level of the analytes that provides measurable response. The LOD was found to be 0.1614 µg/mL & 0.1336 µg/mL & LOQ was 0.4890 µg/mL & 0.4049 µg/mL for Aliskiren & Nicardipine respectively. Data are provided in table 2.

Robustness

Robustness of the planned technique has been evaluated by tiny deliberate changes in the system parameters like rate, mobile phase composition, pH of the mobile part, & temperature. It was found that none of the above parameters caused alteration within the peak space, retention time, & USP tailing by small changes like ±0.1 mL change in flow rate, ±5% change in mobile part, & ±5°C change in temperature. The % RSD was found to be inside the bounds & the tactic was found to be sturdy.

Assay

Analysis of marketed formulation (TAKEMLO tablets, 150 mg Aliskiren & 10 mg of Nicardipine, Novartis, Mumbai, India) was purchased from local market. Five tablets were weighed & average weight was calculated; weight equivalent to five tablets was transferred into a 250 mL volumetrical flask, 60 mL of diluent was additional & sonicated for 25 min, & further the volume was created up with dilutant & filtered. From the filtered solution 0.5 mL was pipetted out into a 10 mL volumetric flask & created up to 10 mL with dilutant. From the resulting resolution 10 µL was injected into HPLC system & peak areas were recorded. The % assay of the marketed formulation was found to be 99.15% for Aliskiren & 99.87% for Nicardipine

Force Degradation Studies

In the present study forced degradation studies were allotted to make sure the effective separation of Aliskiren & Nicardipine from degradation product. Degradation was observed by decreasing the peak areas of the drug substances with same drug molecules of degraded peak areas. The percentage assay of degradation was calculated from the height space obtained in degradation conditions & it had been compared with assay of non-degraded conditions. Acidic & alkali degradation was carried out by treating the sample solution with 2N HCl & 2N NaOH solutions. From the chromatograms, it was found that each the molecules square measure prone to acidic & alkali degradation & proportion assay degradation in both acidic & alkali conditions was found to be inside the bounds. Oxidative degradation studies were performed by treating two hundredth H_2O_2 resolution & keeping it at 60°C for 30 min. The results showed that there was no degradation products shaped. For thermal stress studies the drug solutions were placed in oven at 105°C for 6 h & then injected into HPLC system & photo stress testing was carried out by keeping the drug solutions in ultraviolet light chamber for seven days. In all the conditions the purity of angle is found to be but that of purity of threshold which indicates that the developed technique was stability indicating. The forced degradation studies were performed without intending to establish the degradation product however just to point out that they are not officious with active molecules if any gift.

CONCLUSION:

In the present study, a stability indicating RP-HPLC method has been developed & valid for synchronous estimation of Aliskiren & Nicardipine in pill dose kind. The validated technique has been with success used for stress testing analysis of aliskiren & Nicardipine. The stress testing studies revealed that the tactic was with success utilized to resolve the degraded product from the sample. From the peak purity profile it had been demonstrated that there was no interference of degradation product & the purity of angle were found to be but the purity of threshold. The proposed technique was tested to be selective, accurate, precise, & rapid & it can successfully used for routine analysis of Aliskiren hemifumarate and Nicardipine in bulk and tablet dosage formulation. The method can also be employed in quality control of pharmaceuticals containing Aliskiren and Nicardipine to reduce analytical time.

CONFLICT OF INTEREST:

Authors declares that there is no conflict of interest

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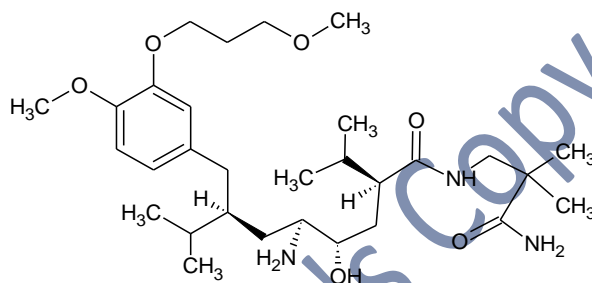
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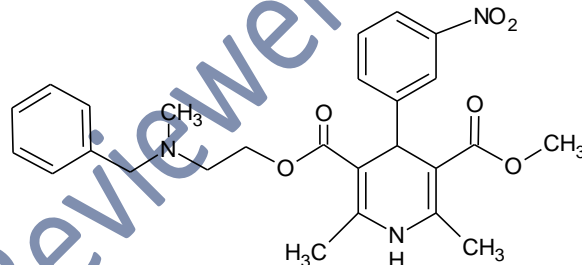

Figure 1: Chemical structure of Aliskiren
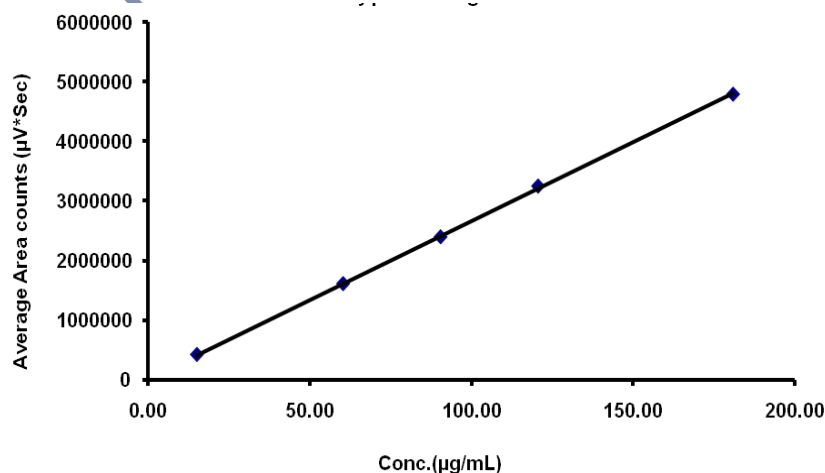

Figure 2: Chemical structure of Nicardipine
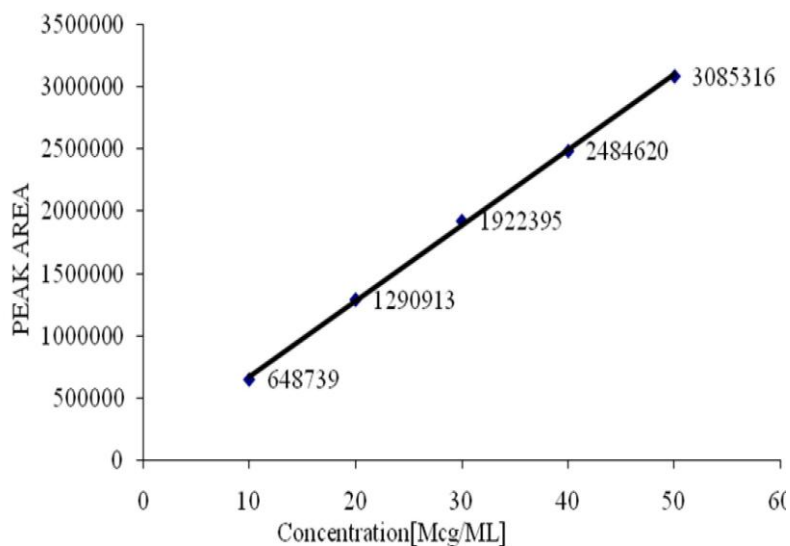

Figure 3: Calibration graph for Aliskiren.

Figure 4: Calibration graph for Nicardipine

Table 1: **Conditions of Chromatography**

Column	C-18 hypersil ODS (150 × four.6 mm, 5 μm)
Sample temperature	30°C
Injection volume	10 μL
Flow rate	1 mL/min
Detector wavelength	237nm
Column temperature	30°C
Detector	UV
Diluent	BUFFER:ACN (40:60)
Mobile phase B	Acetonitrile
Mobile phase A	phosphate buffer
Run time	10 mins.

Table 2: System suitability parameters

Parameter	Result (Aliskiren)	Result (Nicardipine)
Linearity (μg/ml)	30–225	2–15
Correlation coefficient	0.9990	0.9990
LOD (μg/ml)	0.1614 μg/mL	0.1336 μg/mL
LOQ(μg/ml)	0.4890 μg/mL	0.4049 μg/mL