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RESEARCH ARTICLE

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

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Abstract

Objective: 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 and very complex to simplify the methods with increasing sensitivity new methods were developed which are simple, precise, eco-friendly, less time consuming, rapid and fast and economically chief.
Methods: First standard curve was plotted then the method is validated by using recovery studies, linearity, correctness and reproducibility, robustness, ruggedness, detection limit, quantification limits, stability studies etc. The validated technique has been with success used for stress testing analysis of Aliskiren and Nicardipine.
Results: 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 and 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 and M. Phase'. The proposed method was validated as per the ICH and USP guidelines.
Conclusion: 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 and the purity of angle were found to be but the purity of threshold.
Keywords: Aliskiren, nicardipine, RP-HPLC method, tablet dosage forms.

INTRODUCTION

Aliskiren is a novel antihypertensive agent and 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 and angina pectoris. It is a protracted acting metal channel blocker that inhibits the flow of calcium ions into the tube swish muscle and muscular tissue with chemicals Nicardipine is 3-ethyl-5-methyl, 2 [(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4 dihydr-opyridine-6-methyl 3, 5dicarboxylate (Figure 2).

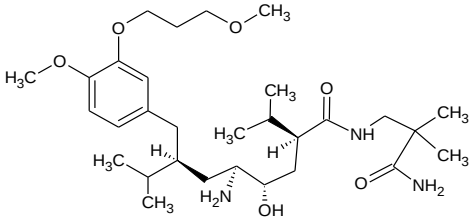

Figure 1: Aliskiren.

Through literature survey reveals that there square measure few analytical ways such as RP-HPLC and UV light ways are rumoured for synchronous estimation of Aliskiren and Nicardipine in pharmaceutical dose forms.

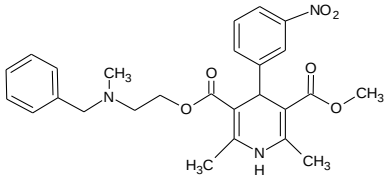

Figure 2: Nicardipine.

But therefore way there's no stability indicating technique rumored. Therefore the gift investigation was allotted to develop new easy, precise, rapid, and cost-effective stability indicating RP-HPLC technique for the synchronous estimation of Aliskiren and 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 propos-ed technique was also successfully used to separate the degraded product from the samples.

MATERIALS AND METHODS

Aliskiren and Nicardipine standards were provided from Spectrum Research Laboratory, Hyderabad, and commercial pill dose kind TEKEMLO was purchased from native market. The HPLC grade acetonitrile and water were purchased from Merck and analytical grade potassium dihydrogen phosphate was purchased from RANKEM. Analytical grade triethylamine, ortho-phosphoric acid, hydrochloric acid, sodium hydroxide, and 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 45 µ and degassed.

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

Preparation of diluents: fuel (HPLC Grade)

Intact pills of Aliskiren Hemifumarate and Nicardipine Besylate taken into 500ml volumetric flask than additional 10ml of water and sonicated until disintegrated. Added concerning 400ml of fuel and sonicated for 45 minutes than created up volume with fuel. Added 5 ml of this resolution in to 50 mm volumetric flask and build up volume with fuel and filtered with 0.45 µm nylon filter. A validated stability indicating RP-HPLC technique development and validation for synchronous estimation of Aliskiren Hemifumarate and Nicardipine Besylate in Pharmaceutical dose kind. The present study describes the soundness indicating RP-HPLC technique for synchronous estimation of Aliskiren hemifumarate and 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 and autosampler. The Empower 2 package was used for signal observation and process. UV chamber has been used for photolytic degradation and 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 and acetonitrile in the ration of 40: 60% v/v and hydrogen ion concentration was adjusted to three with phosphoric acid resolution that was wont to separate the analytes and 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.

Table 1: Conditions of Chromatography.

Column	C-18 hypersil ODS (150 × 4.6 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.

Standard stock solution preparation

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

Sample solution Preparation

Five pills (TAKEMLO tablets: 150 mg Aliskiren and 10 mg Nicardipine) were weighed and 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 addit-ional and sonicated for 25 min; and the amount was created up with dilutant and filtered. From the filtered solution 0.5 ml was pipetted out into a 10 ml volumetric flask and created up to 10 ml with dilutant.

Degradation studies by Force

Forced degradation studies of the drug formulation were carried out by treating the drug samples under stress evoked conditions like acid and base chemical reaction, oxidation, and photo and thermal degradation and interference of the degraded products was

investigated. These studies help to understand the inherent stability characteristic of the active molecules in drug product and the attainable degradation product[7-10].

Degradation studies by acid

To 1 ml stock solution of Aliskiren and Nicardipine, 1 ml of 2N hydrochloric acid was added and refluxed for 30 mins at 60°C. The resultant resolution was diluted to get 150 µg/ml and 10 µg/ml solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the soundness of sample[7-10].

Degradation studies by acid

To 1 ml stock solution of aliskiren and Nicardipine, 1 ml of 2N sodium hydroxide was additional and refluxed for 30 min at 60°C. The sample resolution was ready to get the concentration of 150 µg/ml and 10 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the soundness of sample[7-10].

Oxidation

To 1 ml stock solution of aliskiren and Nicardipine, 1 ml of 20% atomic number 1 peroxide (H_2O_2) 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 and 10 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the soundness of sample[7-10].

Photo stability studies

The photochemical stability of the drug was conjointly studied by exposing the 150 µg/ml and 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/m^2 in photo stability chamber. For HPLC study, the sample resolution was ready to get the concentration of 150 µg/ml and 10 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the soundness of sample[7-10].

Degradation studies by dry heat

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 and 10 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the soundness of the sample[7-10].

RESULTS AND DISCUSSION

A series of trials was conducted with completely different columns like Inertsil ODS and agilent XDB C-18 and C-8 columns with different mobile phases to develop a appropriate RP-HPLC technique for estimation of Aliskiren hemifumarate and Nicardipine besylate in pill dose kind, and finally a typical chromatogram was obtained with phosphate buffer and acetonitrile in the ration of 40: 60% v/v and hydrogen ion concentration was adjusted 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 and analytes were detected with PDA detector at 237 nm. The retention time of Aliskiren and Nicardipine was found to be 3.98 and 5.14 min respectively. Forced degradation studies were also carried exploitation the developed technique and the degraded compounds were effectively resolved from the Aliskiren and Nicardipine in pill dose kind.

Method validation

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

System suitability parameters

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

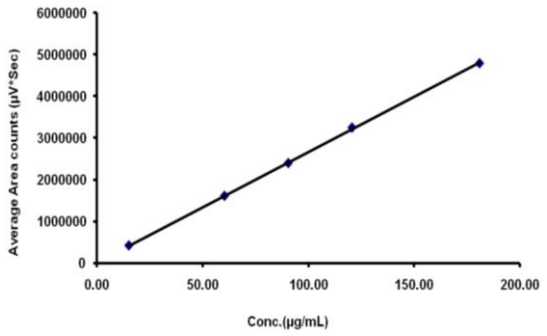

Figure 3: Calibration graph for Aliskiren.

Accuracy

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

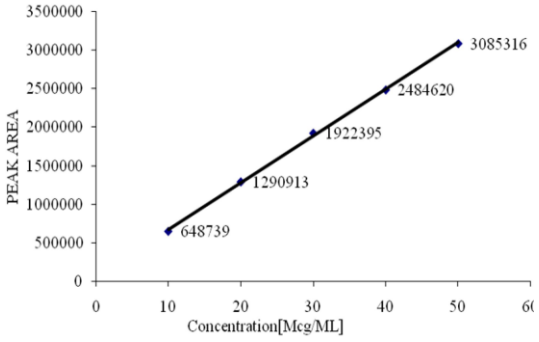

Figure 4: Calibration graph for Nicardipine.

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 and system exactitude studies were allotted by injecting 6 replicates of each standard and take a look at solutions with an equivalent concentration. The % RSD was calculated from the chromatograms and results obtained were inside the bounds of 22 and 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 and from 2 to 15 µg/ml of Nicardipine. All the concentrations were prepared and 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 and Nicardipine (Figure 3, and Figure 4).

Lim. of detection and quantification (LOD and LOQ)

In the present study the LOD and LOQ of Aliskiren and 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 and 0.1336 µg/ml and LOQ was 0.4890 µg/ml and 0.4049 µg/ml for Aliskiren and Nicardipine respectively. Data are provided in Table 2.

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

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, and temperature. It was found that none of the above parameters caused alteration within the peak space, retention time, and USP tailing by small changes like ±0.1 ml change in flow rate, ±5% change in mobile part, and ±5°C change in temperature. The % RSD was found to be inside the bounds and the tactic was found to be sturdy.

Assay

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

Force degradation studies

In the present study forced degradation studies were allotted to make sure the effective separation of Aliskiren and 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 and it had been compared with assay of non-degraded conditions. Acidic and alkali degradation was carried out by treating the sample solution with 2N HCl and 2N NaOH solutions. From the chromatograms, it was found that each the molecules square measure prone to acidic and alkali degradation and proportion assay degradation in both acidic and alkali conditions was found to be inside the bounds. Oxidative degradation studies were performed by treating two hundredth H_2O_2 resolution and 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 and then injected into HPLC system and 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.

CONCLUSIONS

In the present study, a stability indicating RP-HPLC method has been developed and valid for synchronous estimation of Aliskiren and Nicardipine in pill dose kind. The validated technique has been with success used for stress testing analysis of aliskiren and 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 and the purity of angle were found to be but the purity of threshold. The proposed technique was tested to be selective, accurate, precise, and rapid and 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.

AUTHOR'S CONTRIBUTION

Shahul Hameed M: writing original draft, conceptualization, methodology, investigation. **Jat RK:** Writing, review, and editing, supervision, resources. **Indulatha VN:** writing, review, and editing. Final manuscript was read and approved by all authors.

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DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

None to declare.

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