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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION PROTOCOL FOR ALISKIRENHEMIFUMARATE AND IRBESARTAN

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ABSTRACT

Reversed-phase high-performance liquid chromatography method was developed and validated for the simultaneous estimation of Aliskirenhemifumarate and Irbesartan. Chromatographic separation was achieved with a Shimadzu's high performance liquid chromatography C18 column (150X4.6 mm, 5mm) Buffer (Potassium Dihydrogen Orthophosphate pH:3.5):methanol: Acetonitrile with a mobile phase of ratio 45:20:50v/v. The flow rate was set at 1ml/min and the detection wavelength was 271 nm and 236 nm respectively. Quality by design approach was employed for optimization of method parameters like proportion of mobile phase, concentration of buffer and a model highlighting the design space was generated. This developed chromatographic method gave well resolved symmetric peaks. Aliskiren and Irbesartan were eluted at 2.29 and 3.35 min, respectively. This method was validated according to International Conference on Harmonisation Q2(R1) guideline. The method was linear in range of 01 to 05 μg/mL for Aliskirenhemifumarate and 01 to 05 μg/mL of Irbesartan by RP-HPLC and the linearity study showed the regression co-efficient as 0.9994 and 0.996 for aliskiren hemifumerate and irbesartan respectively and The linearity graph of both drugs was obtained in a range of 5 to 25 µg/ ml for Aliskiren hemifumerate and 2 to 10µg/ mL for Irbesartan by UV Spectroscopy. The sample recoveries were in good agreement with the respective label claim, which suggested non-interference from formulation additives in the estimation.

KEYWORDS: Analytical Method Development, RP-HPLC, Simultaneous Equation Method, Aliskiren and Irbesartan.

INTRODUCTION

Aliskiren Hemifumarate (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[1-3] (Fig.1) is an orally active renin inhibitor that is use in hypertension and heart failure. Literature survey reveals Spectrophotometric Methods, RP-HPLC for determination of Aliskiren with other drugs. It is Angiotensin II receptor antagonist[4-7]; 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. Literature survey also reveals Spectrophotometric Methods, HPTLC, RP-HPLC, LCMS, UPLC,[8-12] for determination.

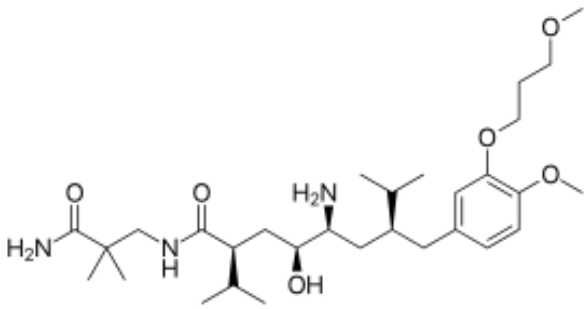

Fig. 1: Aliskiren Hemifumarate.

Irbesartan[13-15] 2-butyl-3-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1,3-diazaspiro[4.4]non-1-en-4-one (Fig 2). Irbesartan is a white to off-white crystalline powder with a molecular weight of 428.5. It is a nonpolar compound and it is slightly soluble in alcohol and methylene chloride and practically insoluble in water. Literature survey reveals that HPTLC, LC, HPLC for determination of content uniformity and simultaneous estimation of Irbesartan is reported, but there is no stability indicating high-performance liquid chromatography (HPLC) method for the determination of Irbesartan from its tablets, as its Pharmaceutical dosage form. The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests (ICH, 1993, 1996). The hydrolytic and the photolytic stability are also required. An ideal stability indicating method is one that quantifies the drug and resolves its degradation products. The aim of the present

work was to develop an accurate, specific, reproducible, and stability indicating method for the determination of Irbesartan in the presence of its degradation products and related impurities as per ICH guideline. Few analytical methods for the estimation of Irebesartan from plasma, and metabolites including HPTLC, HPLC, and GC are reported. To the best of our knowledge, a very few Spectrophotometric methods have been reported. In view of the above fact, some rapid and sensitive analytical methods are in need for its quantitative estimation. The present work describes two simple and accurate spectrophotometric methods for the estimation of Irbesartan in bulk and dosage form.

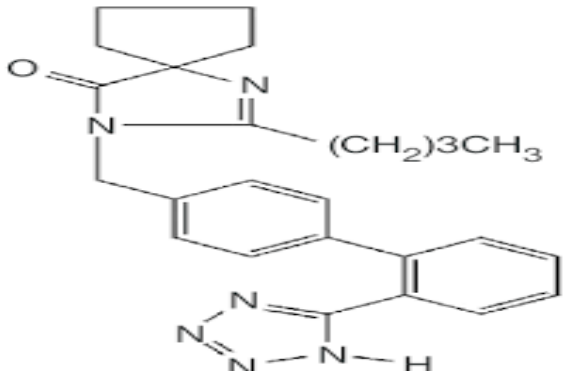

Fig. 2: Irbesartan.

EXPERIMENTAL

Material and Methods

Chemicals and Reagents: Aliskiren and Irbesartan were procured as a gift sample.

Preparation of stock solution (standard) of Aliskiren hemifumarate: 10mg of Aliskiren hemifumerate raw drug was weighed and transferred accurately to the volumetric flask (10ml), thereby dissolving in 1ml DMSO. The volume was later made up with methanol. The prepared solution had a concentration of 1000 µg/ml. An 100 µg/ml of solution was prepared from the standard solution and used as a working standard.

Preparation of working standard (100µg/ml): From stock solution (standard) 1 ml was transferred to 10 ml of volumetric flask and the remaining 09 ml was filled using methanol. From this working standard prepare 10 µg/ml of a solution.

Preparation of stock solution (standard) of Irbesartan: 10mg of raw irbesartan drug was weighed and transferred accurately to a volumetric flask of 10ml thereby dissolving in 1ml DMSO. The volume was later made up with methanol. The prepared solution now has a concentration of 1000 µg/ml. A 100 µg/ml of solution was prepared from the standard solution and used as a working standard.

Preparation of working standard (100µg/ml): From standard stock solution, 1ml was transferred to a volumetric flask of 10 ml and the remaining volume was filled using methanol. From this working standard prepare 10 µg/ml of a solution.

VALIDATION OF DEVELOPED METHOD

Linearity: A calibration curve was plotted of concentration Vs Absorbance. Aliskiren hemifumerate was found to show a linear plot in the range of 5 to 25 µg/ mL and irbesartn in the range of 2 to 10 µg/ mL for the selected wavelength.

Recovery studies (%Accuracy): To ensure the accuracy of the method, recovery studies were performed using a 50% pre-analyzed sample solution, a set of known concentration of standard solution of both the drugs were taken following its recovery studies. The absorbance at respected wavelengths was performed and percentage recovery was calculated.

Precision: these were confirmed using repeatability studies. The repeatability studies were performed by annalyzing the sample solution on constant basis for 3 times. Inter and intraday precision were established by repeating the determination of samples on same and different days respectively.

Ruggedness: Ruggedness of the method was confirmed by the analysis of formulation was done taking help of different analysts. The amount and % RSD were calculated.

Limit of Detection and Limit of quantification: The linearity studies were carried out for six times. The limit of detection and limit of quantification were calculated by using the average of slope and standard deviation of intercept.

Reverse Phase High Performance Liquid Chromatography (RPHPLC)

The technique of chromatography depends on various aspects of drugs like molecular weight, solubility etc. since the drugs selected were polar in nature, reverse phase chromatography can be used for their separation.

Selection of mobile phase and λmax: Many different mixtures of mobile phase were selected in different ratios and their chromatogram was studied. Amongst them buffer, methanol, Acetonitrile was selected as mobile phase as the drugs showed sharp peak. The mobile phase was therefore used to optimize the chromatographic conditions.

Chromatographic conditions optimized: The parameters used for RP-HPLC analysis of aliskiren hemifumerate and irbesartan are:

- **Mode of operation** – Isocratic
- **Stationary phase** – C18 column (150X4.6 mm, 5mm,)

- **Mobile phase** – Buffer (pH:3.5): methanol: Acetonitrile
- **Ratio** – 45:20:50v/v
- **Flow rate** – 1 mL/ min.
- **Run time:** 10 min.
- **Detection wavelength** – 271nm and 236nm.
- **Column Temperature** – ambient
- **Sample volume** – 20 µg/ Ml

Preparation of stock solution (standard) of Aliskiren hemifumarate: 10mg of Aliskiren hemifumerate raw drug was weighed and transferred accurately to the volumetric flask (10ml), thereby dissolving in 1ml DMSO. The volume was later made up with methanol. The prepared solution had a concentration of 1000 µg/ml.

Preparation of stock solution (standard) of Irbesartan: 10mg of raw irbesartan drug was weighed and transferred accurately to a volumetric flask of 10ml thereby dissolving in 1ml DMSO. The volume was later made up with methanol. The prepared solution now has a concentration of 1000 µg/ml.

Preparation of Sample solution: 20µg/mL of a solution of the standard stock of both drugs was prepared using the mobile phase.

Preparation of 20mM Potassium Dihydrogen Orthophosphate (pH=3.5):2.72g of KH_2PO_4 was accurately weighed and transferred to 1000 ml volumetric flask. It was then dissolved and diluted with HPLC grade water and pH was adjusted to 3.5 using orthophosphoric acid.

Linearity and Calibration

Solution concentration of 2 to 10 microgram/ ml of both aliskiren hemifumerate and Irbesartan were prepared and a calibration curve was plotted.

Recovery Studies: In order to ensure the method's reliability, recovery studies were carried out by mixing a known quantity of standard drug solution with a pre analyzed sample formulation, it was mixed thoroughly there by making up to the desired volume. The percentage recovery was then calculated.

LOD and LOQ: The limit of detection and limit of quantification were calculated using the average slope and standard deviation response.

Aliskerin hemifumerate and irbesartan

a. UV-SPECTROPHOTMETRY

i. Calibration: 10 µg/mL of solutions were prepared and were scanned in UV in a range of 200-400 nm. A constant λmax at 271.00 nm was found for Aliskiren hemifumerate and 236.00 nm for Irbesartan. An overlay spectrum of Aliskiren hemifumerate and Irbesartan was also taken (figure 3 and 4). During the overlay(figure 5), it was observed that no two drugs showed absorbance with each other's maximum wavelength. Absorbance stability was also checked at the λmax obtained for respective drugs.

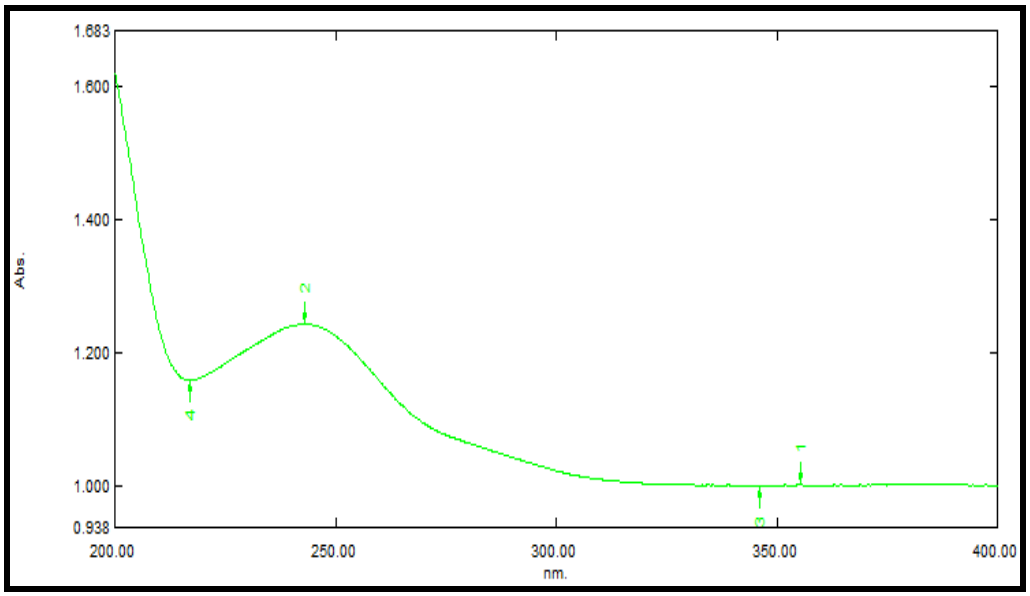

Figure 3: U.V. Spectra- Aliskiren Hemifumerate.

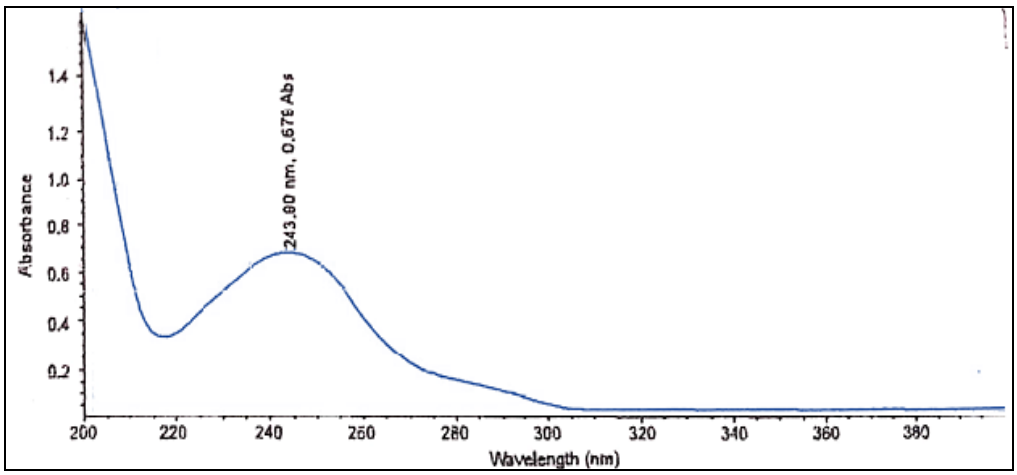

Figure 4: U.V.Spectra- Irbesartan.

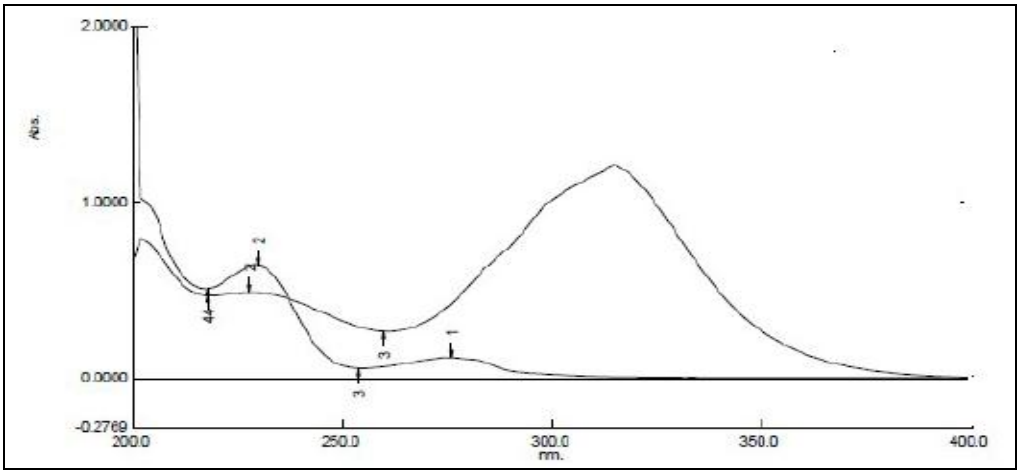

Figure 5: Overlay spectra of aliskiren and irbesartan.

ii. Linearity: The linearity graph of both drugs was obtained in a range of 5 to 25 µg/ ml for Aliskiren hemifumerate and 2 to 10µg/ mL for Irbesartan.

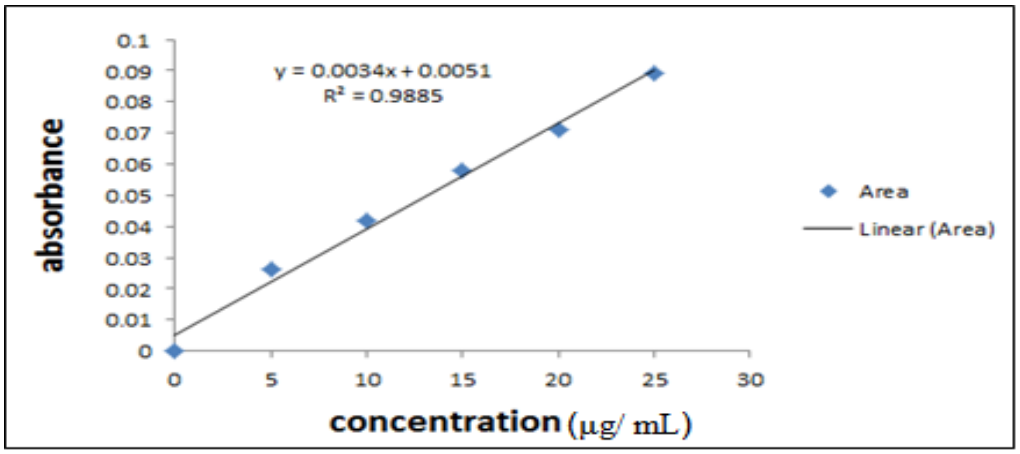

Figure 6: Calibration curve-Aliskiren Hemifumerate.

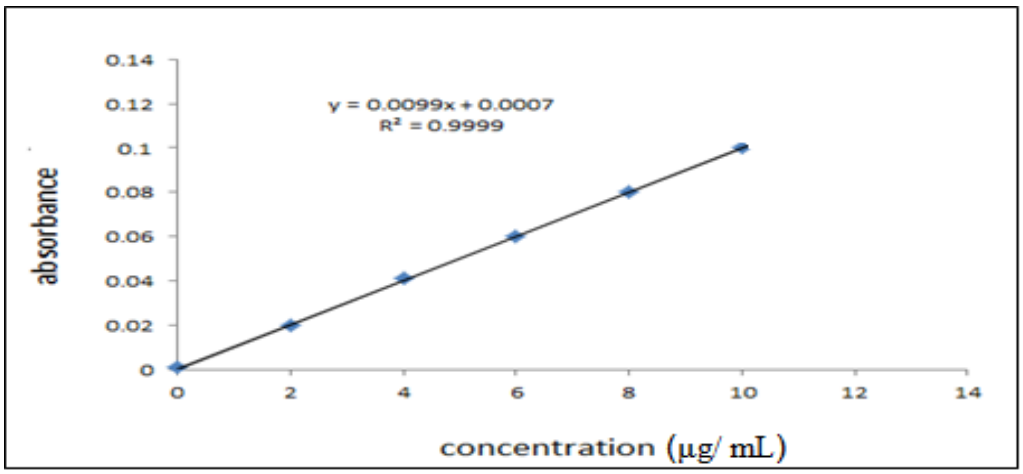

Figure 7: Calibration curve-Aliskiren Hemifumerate.

Table 1: Parameters of UV for aliskiren and irbesartan.

Parameters	Aliskiren hemifumerate	Irbesartan
Wavelength (nm)	271	236
Beer's Law limit (µg/ mL)	5-25	2-10
Sandell's sensitivity (µg /cm2/0.001 A.U)	0.041138	0.034311
Molar absorptivity (L mol–1 cm–1)	21082.81	14923.79
Co-relation coefficient(r)	0.9885	0.9999
Regression Equation (y = mx + c)	y = 0.034x + 0.0051	y = 0.0099x+0.0007
Slope (m)	0.0182	0.0358
Intercept (c)	0.4166	0.2202
LOD (µg/ mL)	0.083	0.049
LOQ (µg/ mL)	2.54	3.11
Percentage RSD	0.0068	0.0021

Precision studies: The precision studies of aliskiren hemifumerate gave %RSD within range for both inter and intraday absorbance.

Table 2: inter-day and intraday study of aliskiren hemifumerate.

Concentration µg/ mL	Intra-day absorbance			Inter-day Absorbance		
	Mean Absorbance	± SD	%RSD	Mean Absorbance	± SD	%RSD
5	0.5947	0.00194	0.7247	0.5931	0.0023	0.7221
10	1.0891	0.006	0.0298	1.1009	0.0057	0.0311
15	1.4989	0.00356	0.0902	1.4499	0.0067	0.0910
20	1.4980	0.00359	0.0905	1.4596	0.0068	0.0911
25	1.4885	0.00358	0.0906	1.4695	0.0069	0.0912

Table 3: inter-day and intraday study of irbesartan.

Concentration µg/ mL	Intra-day absorbance			Inter-day Absorbance		
	Mean absorbance	± SD	%RSD	Mean absorbance	± SD	%RSD
2	0.03133	0.0045	1.8311	0.03122	0.0031	1.8121
4	0.04253	0.0036	1.2321	0.04251	0.0054	1.2314
6	0.05033	0.0041	1.0354	0.05011.	0.0027	1.0369
8	0.05039	0.0045	1.0355	0.06015	0.0018	1.0314
10	0.06031	0.0052	1.0455	0.07014	0.0034	1.7211

Percentage Recovery, LOD and LOQ: The optical properties of the drugs were studied and the results found are: The limit of detection and quantification were determined using linearity studies which was done few times and calculated using slope and standard deviation response.

The percent recovery studies were studied in order to evaluate accuracy of the method, a known amount of drug was added to a pre-analyzed solution containing formulation and the mixture was analyzed thereby calculating the percent recovery.

Table 4: Percent recovery studies-aliskiren and irbesartan.

Drug	%	Amount present µg/ml	Amount added µg/ml	Amount recovered µg/ml	% Recovery	S.D	% RSD
Aliskiren	60	8	6.5	6.4704	99.5446	0.0057	0.0058
	80	8	8	8.0408	100.51	0.0081	0.0082
	100	8	9.5	9.4894	99.8884	0.0064	0.0065
Irbesartan	60	4	2.2	2.1999	99.9954	0.0013	0.0014
	80	4	4	4.0211	100.5275	0.0035	0.0035
	100	4	5.5	5.9874	108.8618	0.0014	0.001

Ruggedness Study: With the help of different analysts, the ruggedness studies were confirmed along with calculation of %RSD.

Table 5: Ruggedness studies of aliskiren and irbesartan.

Drug	Condition	Average % obtained	S. D	%RSD
Aliskiren	Analyst-1	100.38	0.1870	0.1872
	Analyst-2	100.45		
Irbesartan	Analyst-1	100.91	0.1174	0.1171
	Analyst-2	99.87		

II. RP_HPLC METHOD

System Suitability: This was performed in order to confirm the resolution acceptability and repeatability of the current method. System suitability was performed by injecting injections of the standard solution in six replicates. The parameters evaluated were USP tailing, peak area, retention time, theoretical plates and peak asymmetry. With these parameters, percent RSD was also evaluated and were determined to be within limit range. The results are shown in table below

Table 6: System Suitability of aliskiren and irbesartan.

Parameters	Aliskiren	Irbesartan
Retention time (min)	2.29	3.35
USP plate count	3548	4477
USP tailing	1.34	1.23

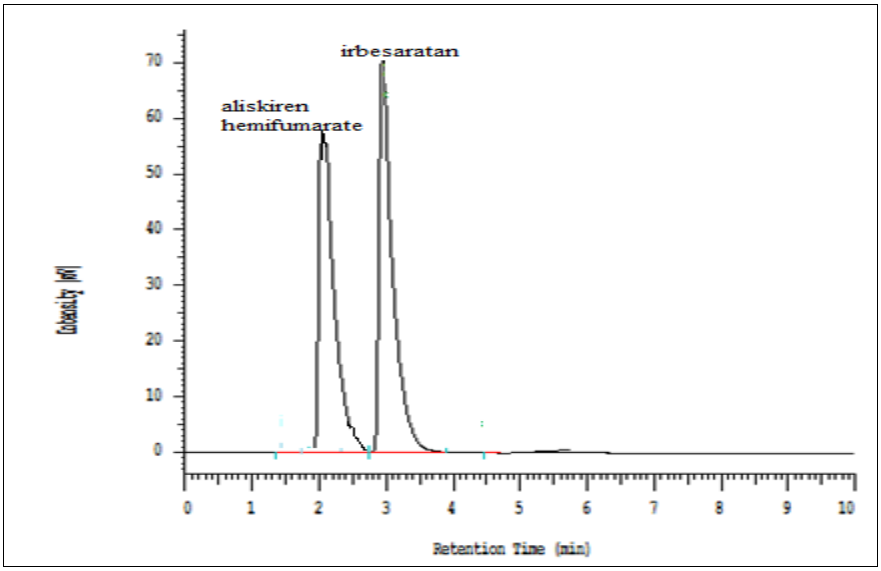

Figure 8: System Suitability of aliskiren and irbesartan.

Robustness: By doing deliberate changes in the system, the robustness of the method was evaluated. The changes were made in mobile phase composition, flow rate, pH of mobile phase and temperature and it was observed and confirmed that no change in parameter altered the method to a great extent. The Percentage RSD was found to be within the expected range and the method was considered as robust. The results are shown in the following table.

Table 7: Robustness study of Aliskiren and Irbesartan.

Sr. no	Parameters	Aliskiren			Irbesartan		
		RT	USPC	TF	RT	USPC	TF
1	Flow rate 0.8ml	2.29	3227	0.24	3.35	4431	0.17
	Flow rate1.2ml	2.21	3536	0.22	3.34	4132	0.21
2.	Temperature 25^0C	2.24	3345	0.19	3.67	4318	0.19
	Temperature 35^0C	2.26	3423	0.28	3.36	4333	0.07
3.	Mobile Phase -5%	2.25	3279	0.19	3.34	4233	0.16
	Mobile Phase +5%	2.27	3638	0.20	3.32	4231	0.14

***PA: peak area; RT: retention time (min); USPC-USP plate count; TF: tailing factor**

Conclusion: Since the methods included, use of chemicals that are economic, and the method can be used in industries as well as a research area for routine analysis of the sample. In method development and validation, the robustness studies of both the pair of drugs proved that the developed method can and will give proper results wherever performed and the ruggedness studies which were performed with help of different analysts also proved the method's precision along with its accuracy. Method development through the reverse phase chromatography technique also gave proper results with all the method validation parameters

like the limit of quantitation and detection, precision, linearity, system suitability along with robustness and ruggedness. Hence, both the method development whether UV spectroscopy or reverse-phase chromatography is feasible and simple for the estimation of the selected drugs and also the chemicals used in the whole process were easily available and were economic.

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