Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(1):290-298
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Stability indicating high performance liquid chromatographic method for the quantification of aliskiren hemifumarate and its related substances

Sampath Kumar Reddy Govindu*[ab], Nagaraju Ch. V. S.[a], Rajan S. T.[a], Eshwaraiah S.[a], Kishore M.[a] and Chakravarthy I. E.[b]

[a]*R&D Center, MSN Laboratories Pvt. Limited, Plot No. 12, Phase-IV, Pashmylaram, Hyderabad, AP, India*
[b]*Rayalaseema University, Kurnool, Andhra Pradesh, India*

ABSTRACT

A reverse phase liquid chromatographic (RP-LC) method was developed for the quantification of the related impurities of Aliskiren Hemifumarate drug substance. The method was optimized using buffer (prepared by dissolving 2.72gr potassium di hydrogen phosphate and 7.0g 1-Octane sulphonoic acid sodium salt anhydrous taken in 1000mL milli-Q-water and then pH was adjusted to 2.7with dilute orthophospharic acid solution) taken along with Acetonitrile 70:30v/v as mobilephase-A, and Acetonitrile: water in the ratio of 90:10 v/v as mobile phase-B. The flow rate was set at 1.0mL min^{-1}, wavelength at 230nm respectively and the column temperature was maintained at 45°C. The capability of stability indicating method developed was demonstrated by studying the degradation products generated during the forced degradation studies under the following conditions i) water hydrolysis, ii) at 75% relative humidity, iii) oxidative, iv) thermal v) photolytic, vi) acid, vii) base, and viii) photolytic degradation. The developed method can be used for the determination of synthetic and degradation impurities in the regular quality control analysis for the drug substance.

Keywords: Aliskiren Hemifumarate, Reverse phase liquid chromatography, Stability-indicating methods, method development, Method validation, Stress conditions, ICH.
**MSNRD Communication No.010*

INTRODUCTION

Aliskiren hemifumarate [(2S,4S,5S,7S)-N-(2-Carbamoyl-2-methylpropyl)-5amino-4-hyd roxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide hemifuma-rate] is an Anti-hypertensive agent. It is used for Management of hypertension (alone or in combina tion with other antihypertensive agents) antagonist (valsartan), or a calcium-channel blocking agent (amlodipine), concomitant use of aliskiren with any of these drugs at maximum recomended dosages produces a greater BP response than does use of each drug alone. Use in combination with an ACE inhibitor or angiotensin II receptor antagonist is contraindicated in diabetic patients and is not recommended in patients with moderate or severe renal impairment. Aliskiren(INN) (trade names Tekturna, US; Rasilez, UK and elsewhere) is the first in a class of drugs called direct renin inhibitors. Its current licensed indication is essential (primary) hypertension.

Aliskiren was co-developed by the Swiss pharmaceutical companies Novartis and Speedel[1-2]. It was approved by the US Food and Drug Administration in 2007 for the treatment of primary hyperte -nsion[3]. In December 2011,

Novartis had to halt a clinical trial of the drug after discovering increased incidence of nonfatal stroke, renal complications, hype Rkale mia, and hypotension in patients with diabetes and renal impairment (ALTITUDE Trial)[4-5]. The biological activity of chiral substances often depends upon their stereochemistry. A large percentage of commercial and investigational pharmaceutical compounds are enantiomers, and many of them show signify cant enantioselective differences in their pharmacokinetics and pharmacodynamics [6-8]. Analysis of the enantiomeric purity of chiral drug candidates has become very important particularly in the pharmaceutical and biological fields, because few enantiomers of racemic drugs have relatively different pharmacokinetic properties and diverse harmacological or toxicological effects [9-12]. apart from this the International Conference on Harmonization (ICH) guide-lines [13-15] emphasizes that the purity and assay of drug susceptible to change during storage, must be determined by using validated stability- indicating methods, which can selectively determine the drug in presence of its process (including the other isomers) and degradation impurities. Renin, the first enzyme in the renin-angiotensin-aldosterone system, plays a role in blood pressure control. It cleaves angiotensinogen to angiotensin I, which is in turn converted by angiotensin-converting enz -yme (ACE) to angiotensin II. Angiotensin II has both direct and indirect effects on blood pressure. It directly causes arterial smooth muscle to contract, leading to vasoconstriction and increaseed blood pressure. Angiotensin II also stimulates the production of aldosterone from the adrenal cortex, which causes the tubules of the kidneys to increase reabsorption of sodium, with water following, thereby increasing plasma volume, and thus blood pressure. Aliskiren binds to the S3bp binding site of renin, essential for its activity[16]. Binding to this pocket prevents the conversion of angiotensinogen to angiotensin I. Aliskiren is also available as combination therapy with hydrochlorothiazide[17]. The empirical formula is $C_{30}H_{53}N_3O_6$. ½ $C_4H_4O_4$ and its molecular weight is 609.8 (free base 551.8)and the chemical structure of Aliskiren hemifumarate is shown in (Fig.1).

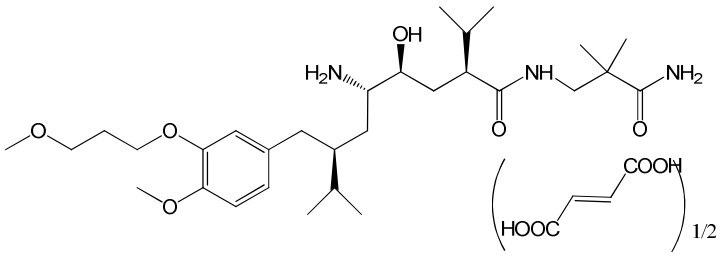

Fig-1: Aliskiren hemifumarate

(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.

Literature survey reveals that few spectrophotometric methods [18] and HPLC methods [19-23] has been reported for the estimation of Aliskiren. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for 'Related substances' of Aliskiren hemifumarate.

MATERIALS AND METHODS

Chemicals and Reagents

Aliskiren hemifumarate and its impurities viz. Michael adduct-1, Michael adduct-2, Hydroxy Aliskiren-1, Desmethoxy Aliskiren, Aliskiren acid, Amino lactone and N-BOC Aliskiren were obtained from MSN Laboratories Private Limited, Hyderabad, India. HPLC-grade of acetonitrile and AR grade of potassium di hydrogen phosphate, ortho phosphoric acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide (30%) were obtained from Rankem, New Delhi, India. Milli Q Millipore (USA) purification system was used to prepare high pure water.

HPLC Instrumentation and Conditions

The method development attempts, forced degradation studies and the method validation was performed in Agilent 1200 series LC systems with a diode array and variable wave length detectors (Agilent Technologies, Waldbronn, Germany). The data were collected and processed using Ez chrom Elite software. The peak homogeneity was studied by using Agilent 1200 series DAD detector.

Chromatographic conditions

The chromatographic separation was optimized in the Symmetry shield RP-18 column with the dimension of 250mm x 4.6 mm and 5µm as particle size. A gradient elution was involved with the buffer (prepared by dissolving 2.72gr potassium di hydrogen phosphate and 7.0g 1-Octane sulphonoic acid sodium salt anhydrous taken in 1000mL milli-Q-water and then pH was adjusted to 2.7with dilute orthophospharic acid solution) taken along with Acetonitrile 70:30v/v as mobilephase-A, and Acetonitrile: water in the ratio of 90:10 v/v as mobile phase-B. The HPLC gradient program was set as (time/% mobile phase- B) 0.01/10, 5/10, 15/20, 40/50, 45/50, 46/10, 53/10. The flow rate of the mobile phase and the column temperature was set as 1.0 mL min^{-1} and 45°C. The detection wave length was optimized at 230 nm, 10µL injection volume. A mixture of equal volumes of Acetonitrile and Water was used as diluent, and diluent used for needle wash purpose.

Preparation of standard solutions:

A mixture of equal volumes of Acetonitrile and Water was used as diluent. A standard solution (Reference solution) 0.0025mg/mL of Aliskiren hemifumarate solution was prepared in the diluent. A stock solution with the blend of Michael adduct-1, Michael adduct-2 (Michael adduct compound is mixture of two diastearomers. Hence two peaks observed. These two impurities are degradent impurities. Michael adduct pure compound is not available. Identified based on LC-MS, RRT's ~0.35 & ~0.38, so Michael adduct impurity not validated), Hydroxy Aliskiren-1, Desmethoxy Aliskiren, Aliskiren acid, Amino lactone and N-BOC Aliskiren was also prepared in diluent for the preparation of system suitability solution (0.15% solution with respect to 2.5mg/mL Aliskiren hemifumarate test concentration).

RESULTS AND DISCUSSION

Method development and optimization

The HPLC method was optimized so as to obtain stability– indicating method that it could resolve degradation impurities from Aliskiren hemifumarate. Different stationary phases with different selectivity were used for the determination of Aliskiren hemifumarate and it's impurities as the initial attempts. However good peak shape with less peak width and the resolution of all the related impurities were achieved satisfactorily in Symmetry shield RP-18 column with the dimension of 250mm x 4.6 mm and 5µm as particle size. A gradient elution was involved a buffer (prepared by dissolving 2.72gr potassium di hydrogen phosphate and 7.0g 1-Octane sulphonoic acid sodium salt anhydrous taken in 1000mL milli-Q-water and then pH was adjusted to 2.7with dilute orthophospharic acid solution) taken along with Acetonitrile 70:30v/v as mobilephase-A, and Acetonitrile: water in the ratio of 90:10 v/v as mobile phase-B. The HPLC gradient program was set as (time/% mobile phase- B) 0.01/10, 5/10, 15/20, 40/50, 45/50, 46/10, 53/10. The flow rate of the mobile phase and the column temperature was set as 1.0 mL min^{-1} and 45°C. The detection wave length was optimized at 230 nm, 10µL injection volume. A mixture of equal volumes of Acetonitrile and Water was used as diluent, and diluent used for needle wash purpose.

The system suitability parameters are resolution between Hydroxy Aliskiren-1 and Aliskiren should not be less than 1.5 and theoretical plates for Aliskiren peak not less than 3000. The developed method is specific for Aliskiren hemifumarate and its degradation products. The structures and chemical names of the impurities are given below:

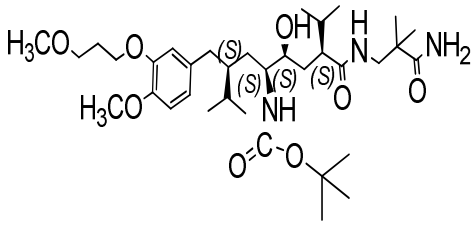

Fig-2: BOC protected Aliskiren

tert-butyl (3S, 5S,6S, 8S)-8-(3-amino-2,2-dimethyl-3-oxopropylcarbamoyl)-6-hydroxy-3-(4-methoxy-3-(3-methoxypropoxy)benzyl)-2,9-dimethyldecan-5-ylcarbamate.

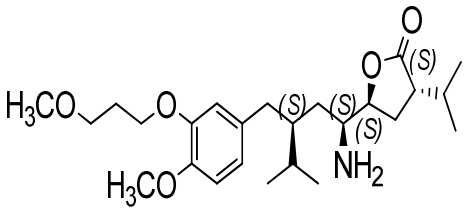

Fig-3: Amino lactone

(3S,5S)-5-((1S, 3S)-1-amino-3-(4-methoxy-3-(3- methoxy propoxy) benzyl)-4-methylpentyl)-3-isopropyl dihydrofuran-2(3H)-one

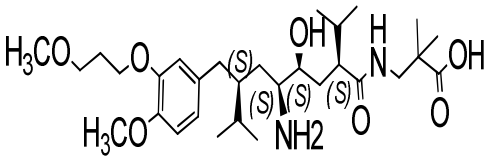

Fig-4: Aliskiren acid

(2S, 4S, 5S, 7S)-N-(2-Carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2, 7-diisopropyl-8-[4-methoxy-3-(3-methoxy propoxy) phenyl]-octanoicacid.

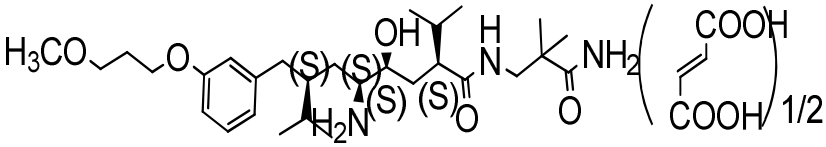

Fig-5: Desmethoxy aliskiren

(2S,4S,5S,7S)-N-(2-Carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[3-(3-methoxypropoxy) phenyl]-octanamide hemifumarate.

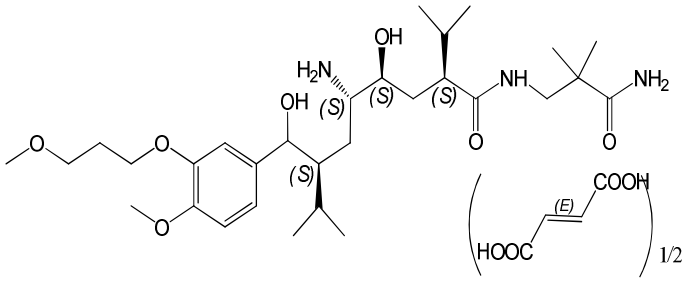

Fig-6: Hydroxy aliskiren

(2S,4S,5S,7S)-5-amino-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-7-(hydroxy(4-methoxy-3-(3-methoxy propoxy) phenyl) methyl)-2-isopropyl-8-methylnonanamide Fumarate

Chromatograms: A typical chromatogram of Blank, mother sample, System suitability solution are given below.

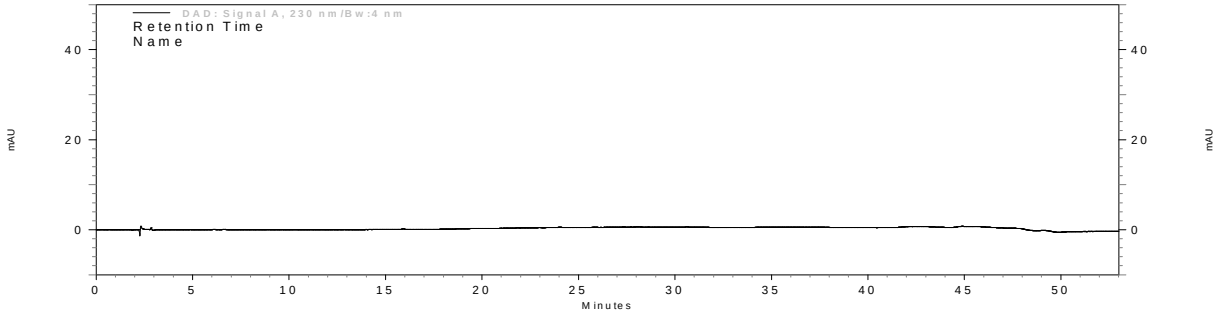

Fig 7: Diluent

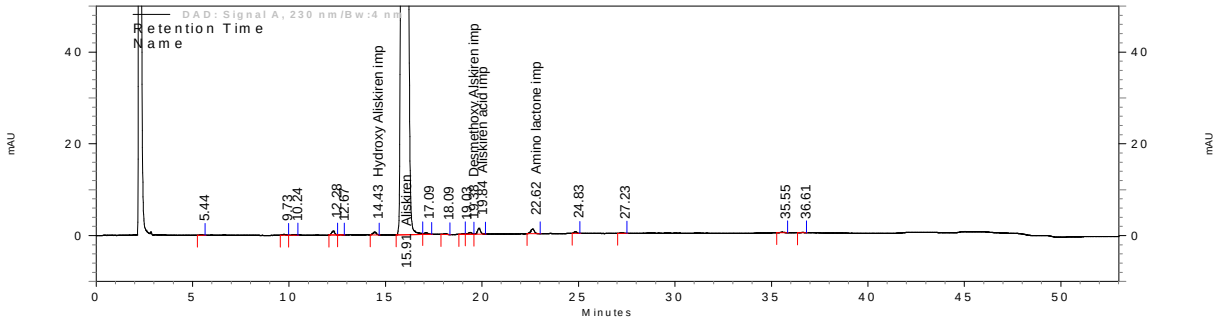

Fig 8: Mother solution

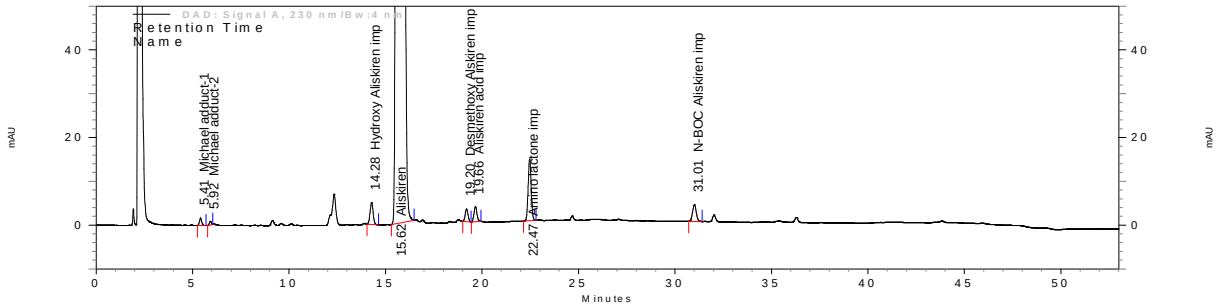

Fig 9: 0.15% all impurities spiked to Aliskiren hemifumarate sample

Method validation Results

The developed method was validated as per ICH guidelines and the results are given (Table I). Stress testing of the drug substance can help to identify the degradants, which in turn help to evaluate the stability-indicating nature of the developed method. The specificity of the developed HPLC method for Aliskiren hemifumarate was determined in the presence of its process and degradation impurities. All the stressed samples of Aliskiren hemifumarate and all degradation impurities were well resolved from one another and from Aliskiren hemifumarate. The analysis was carried out by HPLC with Diod array detector. The chromatographic peak purity tool, applied to Aliskiren hemifumarate and its impurities peaks, demonstrated that all the peaks were pure in all cases conform the absence of other impurities co-eluting in the same retention time and there by signifying the specificity and stability indicating nature of the method. The detection limit (DL) and quantification limit (QL) for Hydroxy Aliskiren-1, Desmethoxy Aliskiren, Aliskiren acid, Amino lactone and N-BOC Aliskiren were determined at a signal to noise ratio of 3:3 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. Precision study was carried at QL level by injecting six times and calculating the percentage of R.S.D of area of Hydroxy Aliskiren-1, Desmethoxy Aliskiren, Aliskiren acid, Amino lactone and N-BOC Aliskiren. Linearity test solutions for purity determination were at six concentration levels from QL to 150 % of the specification level (0.15%). Peak area

versus concentration data was performed by least-squares linear regression analysis. Standard addition and recovery experiments were conducted to determine accuracy of impurities quantitation in bulk drug samples. The study was carried out in triplicate at QL, 50%, 75%, 100%, 125% and 150% level with respect to specification 0.15%. The percentages of recoveries for impurities were calculated(Fumarate peak is coming at ~RT 2.3).

Table-1: Validation data of the developed method

Parameter	Hydroxy aAliskiren-11	Desmethoxy Aliskiren.	Aliskiren acid	Amino lactone	N-BOC Aliskiren	Aliskiren
DL (%)	0.003	0.006	0.006	0.002	0.004	0.003
QL (%)	0.013	0.023	0.014	0.008	0.015	0.010
Method Precision (%RSD)#	0.41	0.49	0.80	0.99	2.34	---
Intermediate precision (%RSD)#		0.94	0.88	0.74	0.77	0.99
Accuracy[a] (% recovery) at:-						
QL	96.7	102.2	96.1	89.6	103.1	
50%	98.1	95.2	96.9	101.0	102.4	
75%	98.7	94.8	96.9	99.5	101.6	
100%	100.0	95.1	98.3	100.5	102.2	
125%	100.8	96.1	98.9	101.5	102.1	
150%	99.8	95.0	98.3	101.2	101.6	

[a] *Carried at QL,50%,75%, 100% and 150% level with respect to specification (0.15%)*

The robustness of developed method was determined by altering experimental conditions purposely and evaluating the resolution between Aliskiren hemifumarate, Hydroxy Aliskiren-1, Desmethoxy Aliskiren, Aliskiren acid, Amino lactone and N-BOC Aliskiren. Flow rate was changed by $\pm$ 0.1 units, pH was varied by $\pm$ 0.2 units and column temperature was studied at 40°C and 50°C instead of 45°C in all above varied conditions the components of the mobile phase were held constant and no significant change (relative error less than 5%) of relative retention time was observed.

Significant changes were not observed in the contents of Hydroxy Aliskiren-1, Desmethoxy Aliskiren, Aliskiren acid, Amino lactone and N-BOC Aliskiren. The stability data confirmed that sample solutions were stable up to 48hrs. The system suitability was established in terms of resolution between Hydroxy Aliskiren-1 and Aliskiren should not be less than 1.5 and theoretical plates for Aliskiren peak not less than 3000, when a 2.5mg/ml Aliskiren hemifumarate solution spiked with 0.15% of Hydroxy Aliskiren-1, Desmethoxy Aliskiren, Aliskiren acid, Amino lactone and N-BOC Aliskiren were injected.

Forced degradation studies

The stability indicating power of the developed method was studied by conducting forced degradation studies on Aliskiren hemifumarate. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed HPLC method for Aliskiren hemifumarate was determined in the presence of its impurities, and degradation products. Forced degradation studies were also performedon relative Humidity study stress at 75% Relative humidity for 10 days. The thermal stress was done at 60 °C for 10 days. The under photolytic stress studies conducted for 50 hours at under sunlight. The photolytic stressed studies were performed for UV Direct (200 watt hours/square meter), UV Indirect (200 watt hours/square meter), Lux direct (1.2 million LUX hours) and Lux in direct (1.2 million LUX hours). Water hydrolysis was performed for 48 hours at 60 °C. The acid stress was performed at 0.5 N HCl at the concentrated sample solution at ambient temperature for 21 hours and base stress was performed at 0.5N NaOH for 24 hours at ambient temperature and the oxidation stress was done using 10% hydrogen peroxide for 48 hours an ambient temperature. Stressed samples of Aliskiren hemifumarate generated were checked for peak purity of by using Agilent diod array detector (DAD). The peak purity is within the limit obtained in all stressed samples, demonstrates the analyte peak homogeneity. The Forced degradation studies results are given below (Table-2).

Table 2. Summary of forced degradation results

S.No	Stressed conditions		Duration	% of Total imp	% of Hydroxy aliskiren-1	% of Desmethoxy aliskiren	% of Aliskiren acid	% of Amino lactone	% of N-BOC aliskiren
1	Normal		---	0.41	0.02	0.04	0.07	0.05	0.00
2	Thermal at 60°C		10 days	1.04	0.02	0.03	0.07	0.16	0.00
4	At 75% Relative Humidity		10 days	0.90	0.02	0.04	0.07	0.36	0.00
5	Under Sunlight		50 hours	2.02	0.02	0.04	0.06	0.05	0.00
6	*Photo Degradation*	UV direct	200watt hours/square meter	0.77	0.02	0.04	0.07	0.06	0.00
		UV indirect		0.38	0.02	0.04	0.06	0.05	0.00
		LUX direct	1.2 million LUX hours	0.64	0.02	0.08	0.07	0.06	0.00
		LUX indirect		0.41	0.02	0.04	0.07	0.06	0.00
7	Acid hydrolysis (0.5N HCl at RT)		After 21hrs	5.08	0.06	0.06	1.31	3.41	0.00
8	Base Hydrolysis (0.5N NaOH at RT)		After 24 hr	7.91	0.02	0.04	5.75	1.08	0.00
9	Oxidation (10% H_2O_2 at RT)		After 48 hrs	1.59	0.04	0.03	0.08	0.11	0.00
10	Water Hydrolysis (at 60°C±5°C)		After 48 hrs	1.22	0.05	0.05	0.08	0.60	0.00

Results of forced degradation studies

Significant degradation was observed in Aliskiren Hemifumarate stressed sample that were subjected to very sensitive in Acid hydrolysis, Base hydrolysis. Sensitive in Peroxide hydrolysis, Sunlight degradation and Water at 60°C±5°C. Slightly sensitive in Thermal, 75% relative humidity, Photo degradation(U.V direct and indirect, Lux direct and indirect). Peak purity test results derived from Diode array detector, confirmed by that Aliskiren Hemifumarate peak is homogeneous and pure in all the analyzed stress samples. The Acid, base, Peroxide, Sun light and Water Degradation chromatograms are given below:

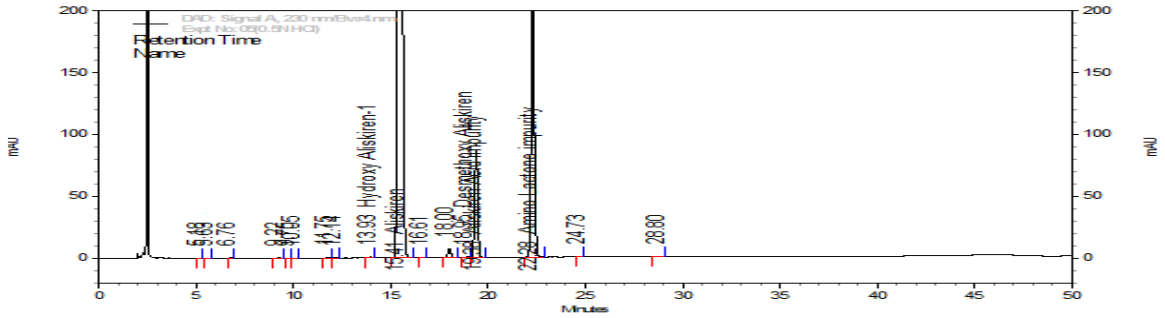

Fig 10: Typical chromatogram of 0.5N HCl degradation solution

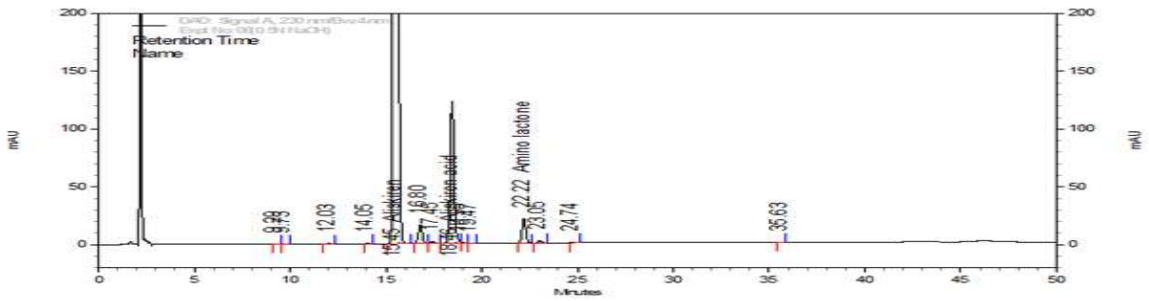

Fig11: Typical chromatogram of 0.5N NaOH degradation solution

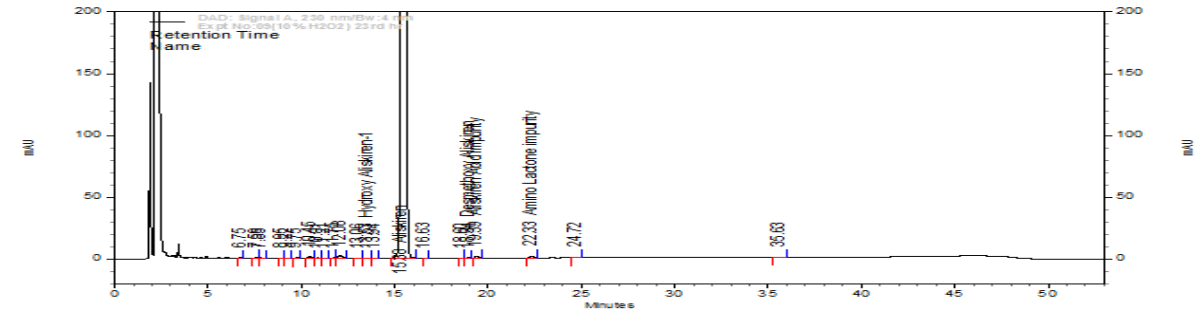

Fig 12: Typical chromatogram of 10% H_2O_2degradation solution at RT

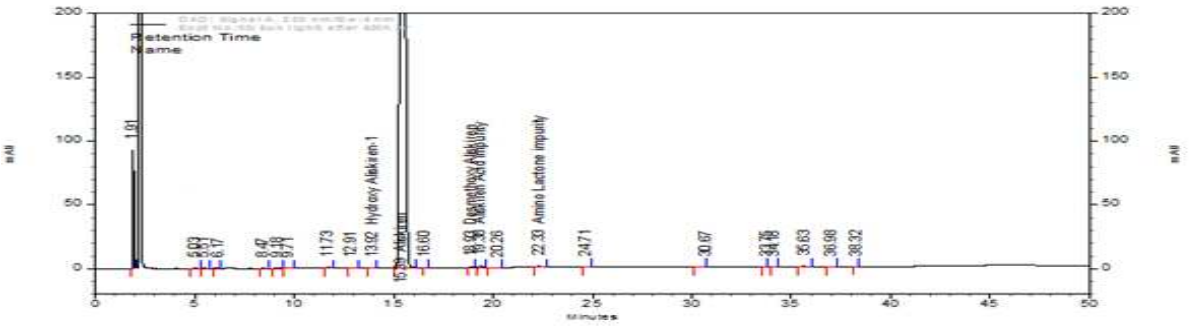

Fig 13: Typical chromatogram of Sunlight degradation solution

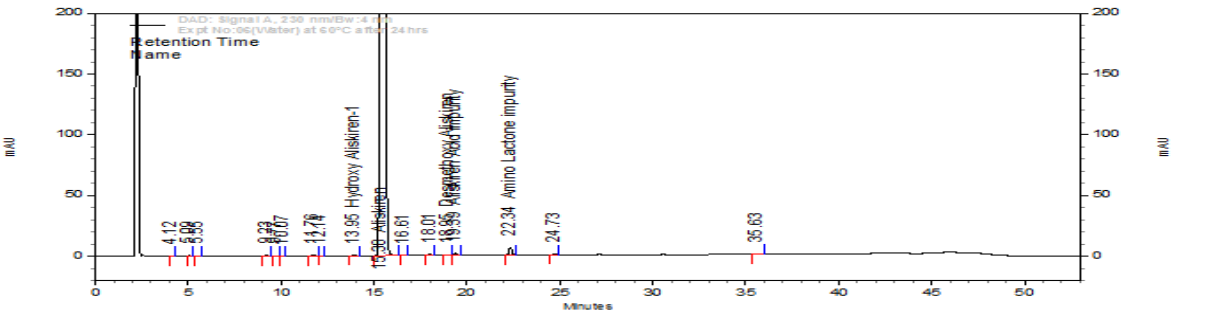

Fig 14: Typical chromatogram of Water degradation solution

Solution stability and mobile phase stability

Solution stability and mobile phase stability provides an indication of its reliability during normal usage during the storage of the solutions used in the method. The solution stability of Aliskiren Hemifumarate was established for 48 hours at room temperature. The solution stability studied by using Aliskiren Hemifumarate sample and injected for every 6 hours. The content of impurities and Aliskiren Hemifumarate were quantified at each interval up to the study period. The mobile phase stability was also established by quantifying the freshly prepared sample solutions against freshly prepared reference standard solutions for every 6 hours up to 48hrs. During the study period the prepared mobile phase was stable up to 48hrs at room temperature.

CONCLUSION

The developed stability-indicating analytical method for related substance determination of Aliskiren Hemifumarate and its impurities is precise, accurate, linear and specific. The validation carried out for the method in accordance with the ICH requirements are satisfactory. The developed method can be used conveniently for the routine analysis of production samples and also to check the stability of bulk samples of Aliskiren Hemifumarate during its storage. The same method can also be attempted for the drug products for the getting the information of impurities and degradation products at lower level.

Acknowledgments
One of the authors Sampath kumar Reddy thanks Dr. Chakravarthy. I.E, for guiding him in his PhD work. The authors wish to thanks Dr. MSN Reddy (MSN laboratories Pvt.Ltd) for providing facilities to do research work.

REFERENCES

[1] Gradman A., Schmieder R, Lins R., Nussberger J., Chiang Y., Bedigian M., Aliskiren, *Circulation*, **2005**, 111 (8): 1012–18
[2] Straessen JA., Li Y., and Richart T., *Lancet*, **2006,** 368 (9545): 1449– 56.
[3] First Hypertension Drug to Inhibit Kidney Enzyme Approved, *CBC*. **2007**-03-06. Retrieved **2007**-03-14.[*dead link*]
[4] Healthzone.ca: Blood-pressure drug reviewed amid dangerous side effects
[5] Parving, Hans-Henrik; Barry M. Brenner, M.D., Ph.D., John J.V. McMurray, M.D., Dick de Zeeuw, M.D., Ph.D., Steven M. Haffner, M.D., Scott D. Solomon, M.D., Nish Chaturvedi, M.D., Frederik Persson, M.D., Akshay S. Desai, M.D., M.P.H., Maria Nicolaides, M.D., Alexia Richard, M.Sc., Zhihua Xiang, Ph.D., Patrick Brunel, M.D., and Marc A. Pfeffer, M.D., Ph.D., Cardiorenal End Points in a Trial of Aliskiren for Type 2 Diabetes for the ALTITUDE Investigators, *NEJM* **2012,** 367 (23): 2204–13.
[6] Stinson S.C., *Chiral drugs, Che. Eng. News*, **2002**, 43, 55–78.
[7] Stinson S.C., *Chiral chemistry, Chem. Eng. News*, **2001**, 45, 57.
[8] Thayer A.M., Interaction yields, *Chem. Eng. News*, **2008**, 12, 20.
[9] Ariens E.J., *Eur. J. Clin. Pharmacol*, **1984**, 26, 663.
[10] Ariens E.J., *Med. Res. Rev*, **1986**, 6, 451.
[11] Ariens E.J., Wuins E.W., *Clin. Pharmacol. Ther*. **1987**, 42, 361.
[12] FDA (Food and drug Administration) Policy Statement for the Development of NewStereoisomeric Drugs, 1992.
[13] International conference on Harmonization Guideline on stability testing of New Drug Substances and products, Q1A (R2), **2003**.
[14] International conference on Harmonization Guideline on Photo stability Testing of New Drug substances and products, Q1B, **1996.**
[15] International conference on Harmonization Guideline on Impurities in New Drug Substances and products, Q3B (R2), **2006.**
[16]Chemistry & Biology : Structure-based drug design: the discovery of novel nonpeptide orally active inhibitors of human renin, *ScienceDirect*, Retrieved **2010**-01-20.
[17] Baldwin CM., Plosker GL, *Drugs* **2009,** 69(7):833-841.
[18] Das P., Patel S., Radhika PP., Subramanyam EVS., Sharbaraya A., *International Journal of Drug Development and Research*, **2012**, 4 (2), 265-270.
[19] Babu KS., JVLNS Rao; Bhargava KV., *Rasayan Journal of Chemistry*, **2011,** 4(2), 285-288.
[20] Sangoi MW., Sangoi MS; Oliveira PR., Secretti LT., CMB Rolim. *Journal of Chromatographic Science*, **2011,** 49,170-175.
[21] Swamy GK., Rao JVLNS., Kumar JMR., Kumar UA., Bikshapathi DVRN., Kumar DV., *Journal of Pharmacy Research*, **2011,** 4(3), 865-867.
[22] Chokshi PV., Trivedi KJ., Patel NS. *International Journal of Chem Tech Research*, **2012,**4 (4), 1623-1627.
[23] Swamy GK., Kumar JMR., Rao JVLNS., Surekha ML., *Journal of Drug Delivery & Therapeutics*, **2012,** 2(5), 162-166.