Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Original Article

QUANTITATIVE SIMULTANEOUS DETERMINATION OF ALISKIREN HEMIFUMARATE AND HYDROCHLOROTHIAZIDE IN COMBINED TABLET FORMULATION BY RP-HPLC

ASHIM K SEN, DHANYA B SEN, RAJESH A MAHESHWARI, R BALARAMAN, A K SETH

Department of Pharmacy, Sumandeep Vidyapeeth University, Piparia, Waghodia, Vadodara 391760, Gujarat, India
Email: ashims01@gmail.com

Received: 11 May 2015 Revised and Accepted: 15 Jun 2015

ABSTRACT

Objective: Development and validation of quantitative simultaneous determination of aliskiren hemifumarate (ALI) and hydrochlorothiazide (HCT) in combined tablet formulation by RP-HPLC.

Methods: The separation of components were achieved on Enable C_{18} column (250×4.6 mm, 5 µm) with a mobile phase consisting of 0.2 %v/v triethylamine in water (pH 6 was adjusted with orthophosphoric acid): methanol (10:90 %v/v) at a flow rate of 1 ml/min was employed. Quantification was achieved with PDA detection at 280 nm. Validation parameters of the proposed method such as specificity, linearity, accuracy, precision and robustness were evaluated according to ICH guidelines.

Results: Linear concentration range was between 1.2-240 µg/ml for ALI and 0.1-20 µg/ml for HCT and correlation coefficient was found to be 0.9995 and 0.9998, respectively. The limit of detection and limit of quantification for ALI was found to be 0.3376 and 1.0230 µg/ml and for HCT 0.0288 and 0.0873 µg/ml, respectively. The results of precision (% RSD<2) studies showed good reproducibility. Recovery study was performed at 50, 100 and 150 % level to check the interferences between analytes and formulation excipients and % recovery was found to be 99.49±0.9868 for ALI and 99.87±0.8556 for HCT. The percentage assay was found to be 100.36±0.9201 and 99.40±0.7624 for ALI and HCT, respectively.

Conclusion: A simple, rapid, cost effective and highly sensitive RP-HPLC method as compared to existing methods for the determination of ALI and HCT in tablet formulation was developed and validated as per ICH guidelines. The method uses simple reagents and sample preparation procedures were minimal. Thus, the proposed method can be applied for routine quality control determination of ALI and HCT in tablet formulation.

Keywords: Aliskiren hemifumarate, Hydrochlorothiazide, RP-HPLC, Tablet formulation.

INTRODUCTION

Aliskiren hemifumarate (ALI) is chemically described as (2S,4S,5S,7S)-N-(2-methylpropyl) 5amino-4-hydroxy-2,7diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octamide hemifumarate, a rennin inhibitor used for the treatment of essential hypertension. Hydrochlorothiazide (HCT), chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfomide 1,1-dioxide is a thiazide diuretic used in the management of hypertension. Chemical structures of both the drugs are shown in fig. 1.

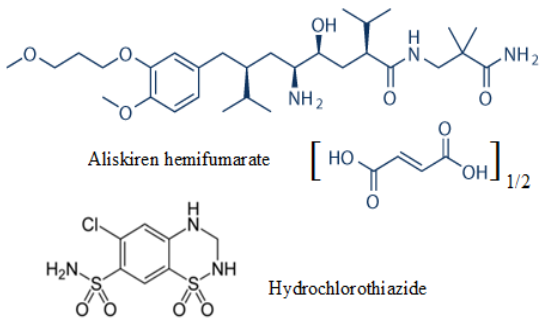

Fig. 1: Chemical structures of ALI (aliskiren hemifumarate) and HCT (hydrochlorothiazide)

Hypertension is one of the most common and powerful risk factors for cardiovascular diseases. Blood pressure control is pre requisite for the management of cardiovascular diseases and complications. More than one medication is required for effective control of blood pressure of the cardiovascular patients. ALI is the first representative of a new class of non peptide, low molecular weight; orally active transition state rennin inhibitor shows the effective control of blood pressure and cardiovascular diseases when combined with HCT, a thiazide diuretic [1-3].

Literature survey revealed various analytical methods for the determination of ALI along with HCT in their pharmaceutical formulation using HPLC [4-15], HPTLC [16-18], spectrophotometry [19] and electrophoresis [20]. Still there was need for a more sensitive method for the determination of ALI and HCT in their tablet dosage form which can cover up the lacuna of some existing methods. Therefore, aim of the present work was to develop and validate a simpler, sensitive, precise, accurate and cost effective RP-HPLC method compared to existing methods for the determination of ALI and HCT in tablet formulation. The advantages of proposed method are as follows; separation was achieved using C18 column (most widely used), 0.2% triethylamine in water (pH 6 with orthophosphoric acid) and methanol as mobile phase, which can be afforded by all the laboratories; fumaric acid was well separated from both the analytes; method describes standard and sample preparation procedure based on the form of analytes under investigation, i.e. aliskiren (13.26 mg of aliskiren hemifumarate is equivalent to 12 mg of aliskiren); wide concentration range with high sensitivity. Moreover, the method can be used for the analysis of ALI and HCT in biological fluids or in pharmacokinetics studies.

MATERIALS AND METHODS

Chemicals and reagents

ALI hemifumarate reference standard was provided by Jubilant Life Sciences Ltd., Noida, Uttar Pradesh, India and HCT was obtained from Glenmark Pharmaceuticals Ltd., Mumbai, Maharashtra, India. Rasilez HCT tablets containing 300 mg of ALI along with 25 mg of HCT were purchased from commercial sources. Methanol and triethylamine used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India and Loba Chemie Pvt. Ltd., Mumbai, India. HPLC grade water was used for all the analyses (Millipore Direct Q3, Millipore India, Bangalore, India).

Apparatus and chromatographic condition

Shimadzu UFLC Prominence (Shimadzu, Kyoto, Japan) equipped with LC-20AD binary pump, SPD-M20A PDA detector and LC

solution as software was used for the analysis. Separation was achieved on Enable C_{18} column (250×4.6 mm i.d., 5 µm particle size, 120 Å). To protect the analytical column a guard column (C_{18}, 120 Å) was used. Isocratic Shimadzu LC system was operated at 25 °C using a mobile phase consisting of 0.2% triethylamine in water (pH 6 was adjusted using 5 %v/v orthophosphoric acid) and methanol (10:90 %v/v) at a flow rate of 1 ml/min and eluents were monitored at 280 nm using PDA detector with 20 µl injection volume.

Preparation of standard solution

The stock solution of HCT was prepared by weighing accurately 10 mg of HCT standard drug which was then transferred to a 10 ml volumetric flask and diluted to 10 ml with mobile phase to get the concentration of the drug 1000 µg/ml. The mixed standard stock solution of ALI and HCT was prepared by weighing accurately, 13.26 mg (13.26 mg of aliskiren hemifumarate is equivalent to 12 mg of aliskiren) of ALI was mixed with 1 ml of HCT standard solution (1000 µg/ml) in to a 10 ml volumetric flask and diluted to 10 ml with mobile phase to get the concentration of the drugs 1200 and 100 µg/ml, respectively.

Preparation of sample solution

Twenty tablets of Rasilez HCT (300 mg of ALI and 25 mg of HCT) were accurately weighed and average weight was calculated. All the tablets were crushed to fine powder and quantity equivalent to 60 mg of ALI and 5 mg of HCT were weighed and transferred to a 50 ml volumetric flask. Flasks was vortexed after adding 30 ml of mobile phase and shaken for 10 minutes and volume was made up to the mark with the mobile phase. Contents were filtered through 0.45 µm membrane filter and suitable aliquots were prepared to get desired concentrations (ALI 120 µg/ml and HCT 10 µg/ml).

Validation of chromatographic method

Analytical method development and validation plays a major role in the different stages of discovery, development and manufacturing of pharmaceuticals. The developed method was validated in accordance with the International Conference on Harmonization [21] guidelines for validation of analytical procedures.

Specificity

In order to determine the specificity of the method, a placebo solution (in-house mixture of all the tablet excipients) was prepared as described in the Handbook of Pharmaceutical Excipients and was analyzed to evaluate the interference among excipients and drugs peak.

Linearity and range

Linearity and range of the method was checked by analyzing mixed standard solutions containing ALI (1.2, 6, 12, 60, 120, 180 and 240 µg/ml) and HCT (0.1, 0.5, 1, 5, 10, 15 and 20 µg/ml) were prepared in mobile phase. Column was equilibrated for 15 minutes with the mobile phase before injecting the solutions. Calibration graphs were plotted using peak areas of standard drugs *vs* concentration. Results were subjected to regression analysis by the least squares method to calculate the values of slope, intercept and correlation coefficient.

Precision

The precision of the method was checked by carrying out repeatability, intra-day and inter-day precision. To check the repeatability of the method a standard mixed solution was injected 6 times and %RSD was calculated. Intra-day precision was carried out by analyzing three replicate injections at two different concentration levels on the same day within the linearity range. Inter-day precision was studied by comparing the results on two different days analyzing three replicate injections at three different concentration levels within the linearity range.

Accuracy

Recovery studies were carried out by the addition of standard drug to pre-analyzed sample solution at three different levels: 50, 100 and 150% to check the accuracy of the method. The resulting solutions were reanalyzed and % recovery was calculated. The result of the accuracy study was assessed based on the percentage of standard ALI and HCT recovered from the formulation.

LOD and LOQ

The limit of detection and limit of quantification of ALI and HCT was calculated using the following equation as per ICH guidelines.

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

Where 5 = the standard deviation of the response, S = the slope of the calibration curve.

Robustness

The robustness of an analytical procedure is refers to its ability to remain unaffected by small and deliberate variations in the method parameters. The method should be robust enough with respect to all critical parameters so as to allow routine laboratory use. Robustness of the method was checked on the basis of slight alteration in the wave length of detection (±2 nm), mobile phase composition (±2 %), flow rate (±0.1 ml/min), buffer pH (±0.5 units) and buffer strength (± 0.1%).

Stability of the solution

Stability of the solutions were checked by observing any changes in the chromatographic pattern compared with freshly prepared solutions by keeping the solutions at room temperature and analyzing at a frequent interval.

System suitability test

System suitability tests were performed to confirm that the instrument was in appropriate condition for the analysis to be performed. Six replicates of the standard solution was injected and chromatograms were recorded to confirm the suitability of the chromatograph.

RESULTS AND DISCUSSION

The objective of the present study was to develop and validate a simple, sensitive and cost effective RP-HPLC method for simultaneous estimation of ALI and HCT in tablet dosage form. Moreover, the other objective of the developed chromatographic method was to obtain better resolution between ALI, HCT and FA (fumaric acid). The chromatographic conditions were optimized to achieve the best resolution and peak shape for both the drugs. The separation of components were achieved on Enable C_{18} column with a mobile phase consisting of 0.2% triethylamine in water (pH 6 was adjusted with orthophosphoric acid) and methanol (10:90 %v/v) at a flow rate of 1 ml/min was employed with PDA detection at 280 nm. The retention time of ALI and HCT was found to be 5.315±0.0264 and 2.824±0.0027 min, respectively, are shown in fig. 2.

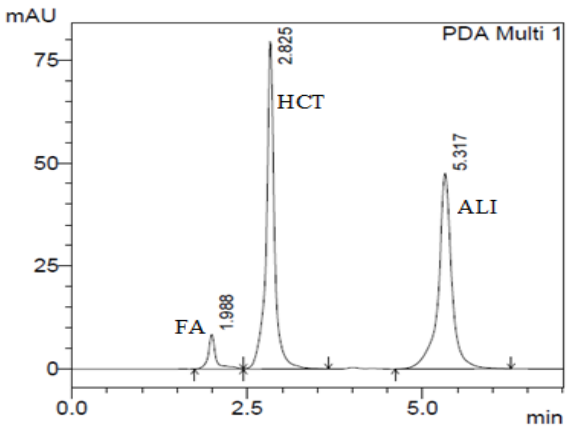

Fig. 2: RP-HPLC chromatogram of ALI (120 µg/ml) and HCT (10 µg/ml)

No interfering peaks were found within the stipulated run time. The developed method was validated as per ICH guidelines. Linearity of the method was established by plotting standard calibration curves between peak area *vs* concentration. ALI and HCT were found to be linear in the concentration range of 1.2-240 µg/ml and 0.1-20 µg/ml, respectively. As both the analytes under investigation obeys beer's law in a wide concentration range, developed method can be applied for formulation as well as bio-analysis. Results were subjected to regression analysis by the least squares method to calculate the values of slope, intercept and correlation coefficient**.** Results of precision studies expressed in % RSD follows ICH guideline acceptable limits, which indicates good repeatability and low inter-day variability, indicating an excellent precision of the developed method. The results of recovery studies ranged from 98-101 % for both the drugs showing the accuracy of the method. This indicates that there is no interference from tablet excipients. The values of LOD and LOQ were found to be 0.3376 and 1.0230 µg/ml for ALI, 0.0288 and 0.0873 µg/ml for HCT, respectively (table 1), proves the sensitivity of the developed method.

Table 1: Summary of validation parameters for the proposed method

Parameters	ALI	HCT
Linearity range (µg/ml)	1.2-240	0.1-20
Correlation coefficient	0.9995	0.9998
Regression equation	y = 5276x+1763	y = 64274x+4229
Precision (%RSD)		
Repeatability of injection (n=6)	0.1917	0.6728
Intra-day (n=3)	0.0942	0.9319
Inter-day (n=3)	0.1220	1.2343
Accuracy		
% Recovery (n=3)	99.49±0.9868*	99.87±0.8556*
% RSD	0.9899	0.7689
Specificity	No interference	
LOD (µg/ml)	0.3376	0.0288
LOQ (µg/ml)	1.0230	0.0873

n = Number of determination, *SD=Standard deviation

The proposed method was checked through all the parameters described earlier under robustness studies, but no significant changes found in retention time, peak area or symmetry of the peaks. Solution stability was performed at room temperature and it was found to be stable up to two days. System suitability tests were performed and the results shows that the parameters tested were within the acceptable range (%RSD<2) and are presented in table 2, which suggests that the method is suitable for an intended use.

Table 2: System suitability parameters

Drugs	Retention time* (% RSD< 2)**	Peak area reproducibility* (% RSD< 2)**	Resolution*	Theoretical plates*	Tailing factor*
ALI	5.346±0.0480* 0.8985**	68258.57±634.53* 0.9296**	9.553±0.1132*	4877±36.20*	0.857±0.0155*
HCT	2.8103±0.0353* 1.2570**	663692.57±5370.09* 0.8091**	4.403±0.0549*	3552±43.58*	0.847±0.0099*

*±SD (n=6), **%RSD

The proposed method was successfully used for the quantitative determination of ALI and HCT in commercial formulation (Rasilez HCT tablet: 300 mg of ALI and 25 mg of HCT). Six replicate determinations were carried out and experimental values are presented in table 3. The percentage assay result obtained ranged from 98-101. Therefore, the proposed method can be successfully applied for the quantitative analysis of ALI and HCT in tablet formulation.

Table 3: Analysis of formulation

Drugs	Labeled amount (mg/tablet)	Amount found (%)*	% RSD*
ALI	300	100.36±0.9201	0.9097
HCT	25	99.40±0.7624	0.7589

*±SD (n=6)

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise, cost effective and highly sensitive as compared to existing methods. The method uses simple reagents and sample preparation procedures were minimal. Therefore, the proposed method can be used successfully for routine analysis of ALI and HCT in tablet dosage form without any interference from the excipients used in the tablet formulation.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacy, Sumandeep Vidyapeeth University, Piparia, Waghodia, Vadodara, Gujarat, India for providing all the necessary facilities to carry out the project work.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Martindale, The complete drug reference. 36th edition. Vol. I. London (UK): Pharmaceutical Press (An Imprint of RPS Publishing); 2009.
2. The Merck Index, An encyclopedia of chemicals, drug and biological. 13th edition. White House Station (NJ), Merck & Co Inc; 2001.
3. Sangoi MS, Sangoi MW, Oliveira PR, Todeschini V, Rolim CMB. Rapid simultaneous determination of aliskiren and hydrochlorothiazide from their pharmaceutical formulations

by monolithic silica HPLC column employing experimental designs. J Liq Chromatogr Relat Technol 2011;34(17):1976-96.

4. Sangoi MW, Sangoi MS, Oliveira PR, Secretti LT, Rolim CMB. Determination of aliskiren in tablet dosage forms by a validated stability indicating RP-LC method. J Chromatogr Sci 2011;49(2):170-5.
5. Ashok S, Varma MS, Swaminathan S. A validated LC method for the determination of the enantiometric purity of aliskiren hemifumarate in bulk drug samples. J Chromatogr Sci 2012;50(9):799-802.
6. Dimitrios K, Eleni K, Irene P. A stability indicating HPLC method for the quantification of aliskiren and hydrochlorothiazide in a pharmaceutical formulation. J AOAC Int 2014;97(6):1519-25.
7. Swamy GK, Sravanthi P, Surekha ML, Kumar JMR, Rao JVLNS. Validated RP-HPLC method for the simultaneous determination of aliskiren, hydrochlorothiazide and amlodipine besylate in bulk and pharmaceutical formulations. Int J Chem Tech Res 2012;4(4):1666-73.
8. Swamy GK, Rao JVLNS, Kumar JMR, Kumar UA, Bikshapathi DVRN, Kumar DV. Analytical method development and validation of aliskiren in bulk and tablet dosage form by RP-HPLC method. J Pharm Res 2011;4(3):865-7.
9. Babu SK, Rao JVLNS, Bhargava VK. A simple and sensitive method for the determination of aliskiren hemifumarate using HPLC-UV detection. Rasayan J Chem 2011;4(2):285-8.
10. Ezzeldin MI, Shokry E, Fouad MA, Elbagary RI. Application of chromatographic and spectrophotometric methods for the analysis of selected antihypertensive combination. Int J Anal Pharm Biomed Sci 2013;2(3):6-15.
11. Ezzeldin MI, Shokry E, Fouad MA, Elbagary RI. Application of chromatographic and spectrophotometric methods for the analysis of aliskiren and hydrochlorothiazide antihypertensive combination. Int J Adv Chem 2013;1(2):13-20.
12. Pachauri S, Paliwal S, Srinivas KS, Singh Y, Jain V. Development & validation of HPLC method for analysis of some antihypertensive agents in their pharmaceutical dosage forms. J Pharm Sci Res 2010;2(8):459-64.
13. Renukapally VK, Rao VU. Stability indicating RP-HPLC method development and validation for simultaneous estimation of aliskiren, amlodipine and hydrochlorothiazide in tablet dosage form. Int J Pharm Pharm Sci 2014;6(1):724-30.
14. Alagar RM, Ragavendra P, Banji D, Rao KNV, Chaithanya Y, Selvakumar D. RP-HPLC method for simultaneous estimation of aliskiren hemifumarate, hydrochlorothiazide and amlodipine in pharmaceutical bulk drugs and tablet dosage form. J Pharm Res 2012;5(8):4580-5.
15. Prathyusha W, Tengli AR, Chandan RS, Gurupadayya BM, Sai TV. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of aliskiren hemifumarate, amlodipine besylate and hydrochlorothiazide in bulk and pharmaceutical dosage forms. IOSR J Pharm Biol Sci 2014;9(1):114-23.
16. Kadukar SS, Gandhi SV, Ranjane PN, Ranher SS. HPTLC analysis of olmesartan medoxomil and hydrochlorothiazide in combination tablet dosage forms. J Planar Chromatogr Mod TLC 2009;22(6):425-8.
17. Bari PD, Rote AR. RP-LC and HPTLC methods for the determination of olmesartan medoxomil and hydrochlorothiazide in combined tablet dosage forms. Chromatographia 2009;69(11-12):1469-72.
18. Shah NJ, Suhagia BN, Shah RR, Patel NM. Development and validation of a simultaneous hptlc method for the estimation of olmesartan medoxomil and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 2007;69(6):834-6.
19. Parmar K, Shah J. Simultaneous estimation of aliskiren and valsartan by ratio spectra derivative spectrophotometry method in their fixed dosage forms. Int J Chem Tech Res 2014;6(2):1268-75.
20. Salim MM, Ebeid WM, El-Enany N, Belal F, Walash M, Patonay G. Simultaneous determination of aliskiren hemifumarate, amlodipine besylate and hydrochlorothiazide in their triple mixture dosage form by capillary zone electrophoresis. J Sep Sci 2014;37(9-10):1206-13.
21. International Conference on Harmonization (ICH). Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva; 2005.