EJAC
Eurasian Journal of Analytical Chemistry

Eurasian J. Anal. Chem. 5(2): 137-144, 2010

ICH guidance in practice: Validated stability-indicating HPLC method for simultaneous determination of Olmesartan medoximil and Hydrochlorothiazide in combination drug products

Vijaya P. Godse*, Ashok V. Bhosale, Yogesh S. Bafana, Dhanaraj D. Borkar,

Pharmaceutical Analysis Department, Seth Govind Raghunath Sable College of Pharmacy, Saswad, Tal- Purandar, Dist- Pune, India. 412 301.

Received: *03 July 2009****; Accepted:*** *24 January 2010*

Abstract

Olmesartan and hydrochlorothiazide were degraded together under different stress test conditions prescribed by International Conference on Harmonization. The samples so generated were used to develop a stability-indicating high performance liquid chromatographic (HPLC) method for the two drugs. The drugs were well separated from degradation products using a reversed-phase (C-18) column and a mobile phase comprising of acetonitrile: phosphate buffer (pH 3.0), which was delivered initially in the ratio of 15:85 (v/v) for 6 min, then changed to 30:70 (v/v) for next 20 min, and finally equilibrated back to 15:85 (v/v) from 20 to 25 min. Other HPLC parameters were: flow rate, 1 mL min^{-1}; detection wavelengths, 258 nm for olmesartan and 224 nm for hydrochlorothiazide and injection volume, 20 µL. The method was validated for selectivity, linearity, precision, accuracy, and specificity. Results were obtained, indicating that the proposed single method allowed selective analysis of both olmesartan and hydrochlorothiazide, in the presence of their degradation products formed under a variety of stress conditions. The developed procedure was also applicable to the determination of instability of the drugs in commercial products.

Keywords:

Olmesartan; Hydrochlorothiazide; Stability; HPLC; Validation

1. Introduction

In recent times, there is an increased tendency towards the development of stability indicating assays [1–3], using the approach of stress testing as enshrined in the International Conference on Harmonization (ICH) guideline Q1AR(2) [4]. Even this approach is being extended to drug combinations [5, 6], to allow accurate and precise quantitation of multiple drugs, their degradation products, and interaction products, if any. The olmesartan, a specific angiotensin II type 1 antagonist, is used alone or with other antihypertensive agents to treat hypertension and hydrochlorothiazide is sodium chloride symporter inhibitor, diuretic [7] and is extensively used for the treatment of hypertension also. There is no stability-indicating assay method reported yet for this combination, developed using the ICH approach of stress testing. Otherwise, there are several HPLC procedures known for the analysis of olmesartan [8–12] and hydrochlorothiazide [13–17] individually, and some methods even exist for simultaneous analysis of the two drugs from dosage forms [18 -20]. Therefore, the focus in the present study was to develop an HPLC stability-indicating method for the combination, by degrading the drugs together under various stress conditions according to ICH. The drugs

* **Corresponding Author**
E-mail: godse_vijaya@rediffmail.com
ISSN: 1306-3057,

were separated from degradation products on a reversed-phase HPLC column and the method was also extended to marketed products. The results are discussed in this paper.

2. Experimental

2.1. Materials

Pure olmesartan and hydrochlorothiazide were obtained as gift samples from Cirex Pharmaceuticals, Andhra Pradesh, India. Combination products containing the two drugs were purchased from local shops. HPLC grade acetonitrile was purchased from Merck Specialties Private Ltd., (Mumbai, India.) Ultra pure water was obtained from a water purification unit (Elga Ltd., Bucks, England). Buffer materials and all other chemicals were of analytical-reagent grade.

2.2. Equipment

The HPLC system consisted of a photo-diode array (PDA) detector (MD-2010 plus), Chrompass software, ver. 1.7.403.1, Pump (PU2080), LC Net II/ ADC (all from Jasco Corporation, Tokyo, Japan). The separations were achieved on a HiQ sil C18W column (250mm×4.6 mm, 10μm) and HiQ sil C18HS **(**250 mm×4.6 mm, 10 μm**)** both from Kya Tech corporation, Japan. The latter was used for intermediate precision studies. A precision water bath equipped with MV controller (Julabo, Seelbach, Germany) was used to carry out selected reactions in solution. Stability studies were carried out in humidity (KBF720, Binder, Germany) and photo stability (KBWF240, WTC Binder, Germany) chambers both set at 40 ^{0}C ± 1 ^{0}C/75 % RH ± 3 % RH. The photo stability chamber was equipped with an illumination bank on inside top consisting of a combination of two black light UV lamps (OSRAM L18W/73) and four white fluorescent lamps (OSRAML 18W/20) in accordance with option two of International Conference on Harmonization (ICH) guideline [21]. The samples were placed at a distance of 9 in. from the light bank. Both fluorescent and UV lamps were put on simultaneously. Thermal stability study was carried out in dry air oven (Innovative DTC 96, New Delhi, India). Other equipments used were sonicator (Spectra lab UCB-30, Mumbai, India), analytical balance (Shimadzu Corporation, Japan).

2.3. Degradation studies

In general, degradation studies were carried out at a concentration of 1 mg mL^{-1} of each drug in the solution. For hydrolysis in water and in 0.1N HCl the solution was refluxed for 1 h. The corresponding reaction in 3% H_2O_2 was carried out for 1 h at room temperature. In 0.01N NaOH solution was refluxed for 3 hr. Degradation was also carried out in solid state by exposing pure drugs to dry heat at 50 ^{0}C for 20 days, and in dark and photo stability chambers for 5 days. Samples were withdrawn periodically and subjected to analysis after suitable dilution.

2.4. Development of method

HPLC studies were carried out on all the reaction solutions individually, and on a mixture of the solutions in which decomposition was observed. The separations were achieved by gradient elution using acetonitrile: phosphate buffer (10 mM, potassium dihydrogen phosphate, and pH 3.0) as the mobile phase. It was filtered through 0.45 μm nylon filter and degassed before use. The injection volume was 20 μl and mobile phase flow rate was 1 mL min^{-1}. The detection of olmesartan and hydrochlorothiazide was carried out at 258 nm and 224 nm respectively.

2.5. Validation of the method

The method was validated for linearity, precision (inter-day, intra-day and intermediate precision), accuracy and specificity. Standard plots were constructed for both olmesartan and hydrochlorothiazide in the range of 100–500 μg mL^{-1} and 50-500 μg mL^{-1} respectively. The experiment was repeated thrice on the same day and additionally on two consecutive days to determine intra- and inter-day precision, respectively. The intermediate precision of the method was determined by repeating the experiment on two different columns. Accuracy was determined by fortifying the mixture of degraded solutions with three known concentrations of the drugs. Further, specificity of the method was assessed by study of the resolution factor of the drug peaks from each other. The selectivity was determined by checking peak purity of the drug peaks, using a PDA detector.

2.6. Application of the developed method to the marketed FDC formulations containing olmesartan and hydrochlorothiazide

The developed method was extended to the analysis of two marketed tablet formulations containing olmesartan and hydrochlorothiazide together. The contents of the tablets were dissolved in 100 ml water. The resultant solution was filtered through 0.45μm nylon and analyzed by the developed method.

3. Results and discussion

3.1. Degradation behavior

HPLC studies on the combination under different stress conditions indicated the following degradation behavior.

3.1.1. Acidic condition

The combination showed sufficient degradation by refluxing for 1 h, in 0.1N HCl. Hydrochlorothiazide showed higher degradation as compared to olmesartan. The major degradation products formed were at retention times (RTs) 2.6, 3.6 4.2, 6.7 and 15.9 min.

3.1.2. Neutral (water) degradation

Sufficient degradation was observed upon refluxing the combination for 3 h. The degradation products appeared at RTs 6.7 and 15.9 min.

3.1.3. Degradation in alkali

Combination of the drugs were found to be highly labile to alkaline hydrolysis in 0.01N NaOH at 40 ^{0}C and most of the drug decomposed within 3 h. The major products appeared at RTs 3.6, and 15.9 min.

3.1.4. Oxidative degradation

The drugs showed sufficient degradation when the combination was degraded in 1 3 % H_2O_2 for 1 h. The major degradation products appeared at RTs 6.7, 15.9 min.

3.1.5. Solid state studies

Solid state studies showed that the combination was unstable in dark, light as well as thermal conditions. Sufficient degradation was observed in accelerated conditions in dark and light after 5 days. The degradation in light was more as compared to dark conditions which is observed at 6.7, 15.9 and 16.9 min. On the other hand, enough degradation was observed

when the combination was exposed to dry heat at 50 ^{0}C for 20 days. The major degradation products resolved at 3.6 and 15.9 min.

3.2. Development and optimization of the stability-indicating HPLC method

A gradient method was found necessary to optimize the separation of major degradation products formed under various stress conditions. The best resolution was achieved with initial run of acetonitrile: phosphate buffer (pH 3.0) in the ratio of 15:85 (v/v) for 6 min, which was then changed to 30:70 (v/v) for next 20 min and finally equilibrated back to the same ratio of 15:85 (v/v) from 20 to 25 min. The method worked well with the mixture of degradation solutions. Fig. 1-3 shows the chromatographic resolution of the blank, mixture of stressed samples, and a formulation, respectively.

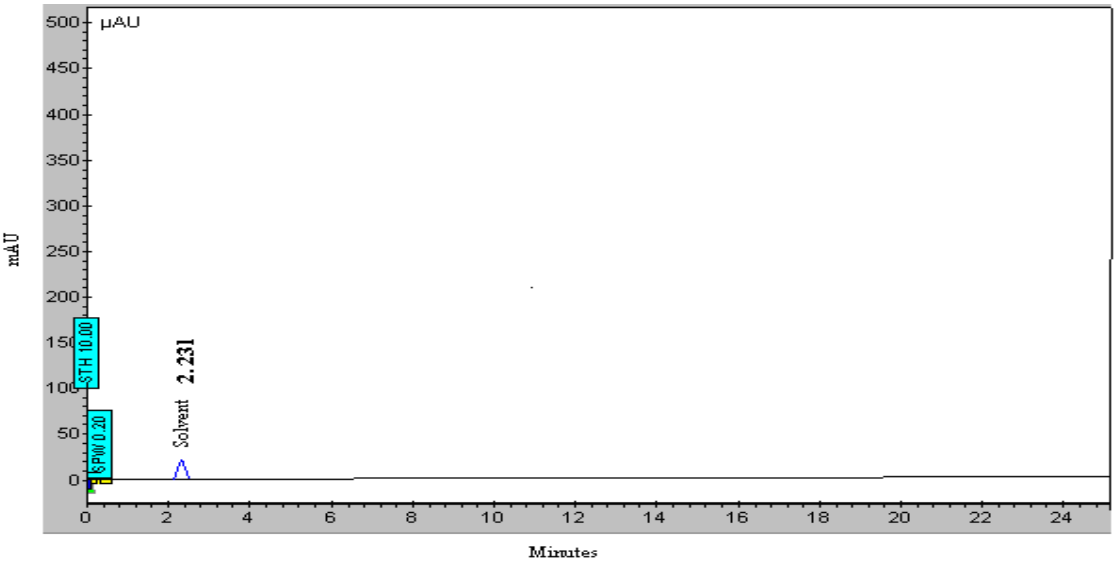

Fig. 1. Chromatogram's showing resolution of components in blank (I: formed in HCl, II: formed in HCl NaOH and thermal; III: formed in HCl; IV: formed in HCl, H_2O, H_2O_2 and light. V: formed in light, thermal conditions, H_2O_2, HCl, NaOH and H_2O; VI: formed in light)

3.3. Validation of the developed stability-indicating method

The linearity could be established for both the drugs olmesartan and hydrochlorothiazide in the range of 100–500 µg/ml and 50-500 µg/ml respectively. (See Table 1).Table 2 lists the relative standard deviation (R.S.D.) data obtained on analysis of the samples on the same day (n = 3) and on consecutive days (n = 3). As evident, the R.S.D. values were < 2 % for intra- and inter-day studies, respectively, demonstrating that the method was sufficiently precise. Even intermediate precision was established for the method, as almost similar resolution was observed on repeating the experiment on two different reversed phase HPLC columns (Table 3). Table 4 shows that recovery of the added drug, obtained from the difference between peak areas of unfortified samples and fortified samples, was satisfactory at all the tested concentrations. As shown in Fig. 2, the method had sufficient specificity and selectivity as the two drugs were well separated from each other, with the resolution factor of > 2 in all cases. Both the drug peaks were pure, which was proved through PDA purity studies. Data of peak purity index values are listed in Table 5.

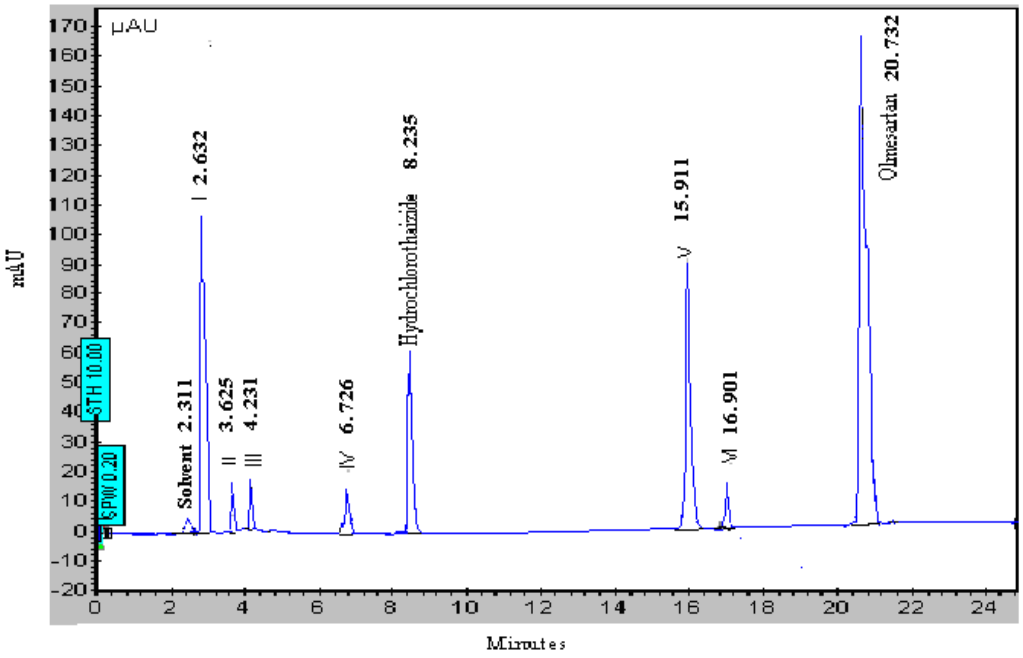

Fig. 2. Chromatogram showing resolution of components in mixture of stress samples. (I: formed in HCl, II: formed in HCl NaOH and thermal; III: formed in HCl; IV: formed in HCl, H_2O, H_2O_2 and light. V: formed in light, thermal conditions, H_2O_2, HCl, NaOH and H_2O; VI: formed in light.)

Table 1. Linearity data for olmesartan and hydrochlorothiazide (n=3)

Drug	Regression parameters		
	Range (µg mL^{-1})	Equation of regression line	R^2 value
Olmesartan	100-500	y =0.0592x - 0.7429	0.9939
Hydrochlorothiazide	50-500	y =0.1415x + 0.2189	0.9921

Table 2. Intra-day and inter-day precision studies (n=3)

Drug	Range (µg mL^{-1})	Intra-day precision	Inter-day precision
		Found ± S.D. (µg mL^{-1}), R.S.D. (%)	Found ± S.D. (µg mL^{-1}), R.S.D.
Olmesartan	100	100.1 ± 0.1,1.6	99.1 ± 0.1,1.6
	200	200.1 ± 0.1,1.6	199 ± 0.17,1.39
	250	249.7 ± 0.4,3.05	251 ± 0.23,1.6
	300	299.8 ± 0.3,1.84	301 ± 0.26,1.34
	400	401± 0.29,1.23	399.2 ± 0.23,0.90
	500	499.7 ± 0.2,0.6	499.2 ± 0.21,0.71

Table 2. (continued)

Hydrochlorothiazide	50	49.7 ± 0.25,1.03	49.8 ± 0.1,1.23
	100	100.2 ± 0.26,1.83	100 ± 0.15,1.02
	200	200.1 ± 0.26,1.88	201 ± 0.07,0.002
	300	299.1 ± 0.36,0.9	299 ± 0.74,1.94
	400	400 ± 0.26,0.4	401 ± 0.1,0.16
	500	499.7 ± 0.3,0.41	501 ± 0.07,0.09

Table 3. Intermediate precision studies

Column	Retention time (min.)	
	Olmesartan	Hydrochlorothiazide
HiQ sil C-18 W, (10µm,25×4.6mm id)	20.7	8.2
HiQ sil C18HS (10µm,25×4.6mm id)	20.5	8.0

Table 4. Recovery studies of olmesartan and hydrochlorothiazide

Drug	Added concentration (µg/mL)	Measured concentration (µg/mL)	% recovery	Mean % recovery
Olmesartan	50	50.2	100.4	
	100	101	101	100.80 ± 0.64
	200	200	100	
Hydrochlorothiazide	50	50.3	100.6	
	100	99.7	99.7	100.26 ± 0.49
	200	201	100.5	

Table 5. PDA peak purity parameters for olmesartan and hydrochlorothiazide under various stress conditions

Drug products	Peak purity index
Olmesartan	1.000
Hydrochlorothiazide	0.9999

3.4. Application of the developed method to marketed FDC formulations containing olmesartan and hydrochlorothiazide

The developed method was used to analyze marketed formulations containing the two drugs. The marketed formulations were subjected to accelerated condition (40 ^{0}C temp,75% RH) for three months as per ICH guideline but no degradation was seen and hence it indicates that packaging material is of good quality and excipients are compatible with drug substances. A clear resolution of the drugs was achieved even for all formulations tested, with

no interference from excipients. In almost all the cases, chromatographic pattern was similar to the one shown in Fig. 3. This indicated that the method could be extended for the study of available drug content 1 in commercial products. (Table 6).

Table 6. Analysis of formulations containing olmesartan and hydrochlorothiazide Combination

Formulation	Label claim (%)	
	Olmesartan	Hydrochlorothiazide
Olmecip	100.2	99.7
Olmat-H	100.1	98.9

(*n*=3)
(Key: blister pack containing 20 mg of olmesartan and 12.5 mg of hydrochlorothiazide.)

4. Conclusion

This study presents a simple and validated stability-indicating HPLC method for simultaneous estimation of olmesartan and hydrochlorothiazide in the presence of degradation products. The method could be applied with success even to the analysis of marketed products, as no interference was observed due to excipients or other components present.

5. Acknowledgement

The authors are grateful to University Pune of for providing grant for this project, to Cirex Pharmaceuticals, Andhra Pradesh, India. for providing gift samples of Olmesartan medoximil and Hydrochlorothiazide and also to Seth Govind Raghunath Sable College of Pharmacy, Saswad for providing facilities to carry out the work.

References

1. Singh S, Singh B, Bahuguna R, Wadhwa L and Saxena R (2006) Stress degradation studies on ezetimibe and development of a validated stability-indicating HPLC assay. J. Pharm. Biomed. Anal. 41: 1037.
2. Mohammadi A, Haririan I, Rezanour N, Ghiasi L and Walker R B (2006) A stabilityindicating high performance liquid chromatographic assay for the determination of orlistat in capsules J. Chromatogr. A 116: 153.
3. Ivana I, Ljiljana Z and Mira Z (2006) A stability indicating assay method for cefuroxime axetil and its application to analysis of tablets exposed to accelerated stability test conditions. J. Chromatogr. A 119: 209.
4. Grosa G, Grosso E D, Russo R and Allegrone G (2006) Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup. J. Pharm. Biomed. Anal. 41: 798.
5. Mishal A and Sober D (2005) Stability indicating reversed-phase liquid chromatographic determination of metronidazole benzoate and diloxanide furoate as bulk drug and in suspension dosage form. J. Pharm. Biomed. Anal. 39: 819.
6. Al Sharef O, Feely J, Kavanagh P V, Scott K R and Sharma S C (2007) An HPLC method for the determination of the free cortisol/cortisone ratio in human urine. Bio. Chromatogr. 21: 1201.

7. Liua D, Hua P, Matsushimab N, Lia X and Li J J (2007) Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandom mass spectroscopy. J. Chromatogr. B 1: 190.
8. Sultana N, Aryane M S, Ali S S and Sajid S (2008) Simultaneous determination of olmesartan, medoxomil and irbasartan and hydrochlorthiazide in plasma formulation andhuman serum using high performance liquid chromatography. Chin. J. Chromatogr. 26:544.
9. 9. Sagrili O, Onal A, Toker S E and Sensoy D (2007) Simultaneous HPLC analysis of olmesartan and hydrochlorthiazide in combined tablet and in vitro dissolution studies. Chromatogr. 66: 213.
10. Shah N J, Suhagia B N, Shah R R and Patel N M (2007). HPTLC Method for the Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Tablet Dosage Form. Ind. J. Pharm. Sci. 16: 834.
11. Menon G N and White L B (2006) Simultaneous determination of hydrochlorothiazide and triamterene in capsule formulations by highperformance liquid chromatography . J. Pharm. Sci. 70: 211.
12. Papadoyannis I N, Samanidou V F, Georga K A, Georgarakis E (1998) Solid-Phase Extraction Study and Photodiode Array RP-HPLC Analysis of Xanthine Derivatives in Human Biological Fluids. J. Liq. Chromatogr. & Related Techn. 21: 1671.
13. Kartal M and Erk N (1999) Simultaneous determination of hydrochlorothiazide and amiloride hydrochloride by ratio spectra derivative spectrophotometry and highperformance liquid chromatography. J. Pharm. Biomed. Anal. 19: 477.
14. Obando M A, Estela J M, Cerda V (2008) Simultaneous determination of hydrochlorothiazide and losartan potassium in tablets by high-performance low-pressure chromatography using a multi-syringe burette coupled to a monolithic column. J. Anal. Bio. Chem. 391: 2349.
15. Shah S H, Shah C R, Shah N J, Patel N M and Suhagia B N (2007) Development and validation of HPTLC method for the estimation of Sumatriptan in tablet dosage form. 59th Indian Pharmaceutical congress, F-10, 392.
16. Sagrili O, Onal A, Toker S E and Sensoy D (2007) Simultaneous HPLC analysis of olmesartan and hydrochlorthiazide in combined tablet and in vitro dissolution studies. Chromatogr. 66: 213.
17. Obando M A, Estela J M, Cerda V (2008) Simultaneous determination of hydrochlorothiazide and losartan potassium in tablets by high-performance low-pressure chromatography using a multi-syringe burette coupled to a monolithic column. J. Anal. Bio. Chem. 391: 2349.
18. Shah S H, Shah C R, Shah N J, Patel N M and Suhagia B N (2007) Development and validation of HPTLC method for the estimation of Sumatriptan in tablet dosage form. 59th Indian Pharmaceutical congress, F-10, 392.
19. Stone A (2005) Stability indicating HPLC method for hydrochlorthiazide. American J. Hyper. 18: 73.
20. ICH, Q1 (B) (1999) Stability testing: Photo stability testing of new drug substances and products, in: International Conference on Harmonization, IFPMA, Geneva, 199.