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Optimization and Validation of Rp - Hplc Stability Indicating Method for Determination of Olmesartan Medoxomil and Its Degraded Product

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Abstract: Stability testing of an active substance or finished product provide evidence as to the quality that it remains acceptable up to the stated period under storage condition as on label. With this objective a stability indicating high performance liquid chromatographic method has been established for analysis of Olmesartan medoxomil in the presence of degradation products. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis, thermal degradation. Extensive degradation was found in acid medium and alkaline medium. Minimum degradation was found in thermal degradation while there was no degradation found in photolytic condition. Successful separation of a drug from degradation product formed under stress condition was achieved on C18 column using methanol: water (60:40, v/v), pH 3.75 adjusted with 10mM o-phosphoric acid mobile phase. Flow rate was 1 ml $min^{-1}$and the detector was set at wavelength of 270 nm. The method was validated for linearity, range, precision, and accuracy, limit of quantification and limit of detection. Because method effectively separates the drug from their degradation products, it can be used as stability indicating method.

Keywords: Olmesartan medoxomil; stress degradation; stability indicating method; HPLC.

1. Introduction

Olmesartan medoxomil (5-methyl-2-oxo-2H-1,3-dioxol-4-yl) methyl4-(2-hydroxypropan-2-yl) -2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate (Figure 1) is a potent antihypertensive works by blocking the binding of angiotensin II to the AT_1 receptors in vascular muscle [1, 2].

Literature survey reveals that there are analytical methods available for determination of Olmesartan medoxomil from biological matrix, bulk drug and dosage form [3, 4, 5] but there is no official method available for the studying the impurities and related substances in Olmesartan medoxomil. Moreover there is no validated stability indicating analytical method for the determination of Olmesartan medoxomil in the presence of degraded product in bulk drug. Hence attempt was made to develop a stability indicating HPLC method for the degraded substances determination [5, 6].

Keeping in view of susceptibility of Olmesartan medoxomil under variety of condition, it was felt that a HPLC method of analysis that separates the drug from the degradation products which

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are formed under ICH suggested condition such as hydrolysis, oxidation, photolysis, and thermal degradation would be remarkable interest [7, 8, 9]. These studies serve to give information on drugs inherent stability and help in the validation of analytical method to be used in the stability studies. Therefore, the objective of research work was to study the degradation of Olmesartan medoxomil under different ICH stress condition and to establish accurate, specific, reproducible stability indicating HPLC method. [7, 8].

This paper deals with the forced degradation of Olmesartan medoxomil under stress condition like acid hydrolysis, alkaline hydrolysis, oxidation, photolysis, and thermal. It also deals with the validation of the developed method for the accurate quantification of degradation product.

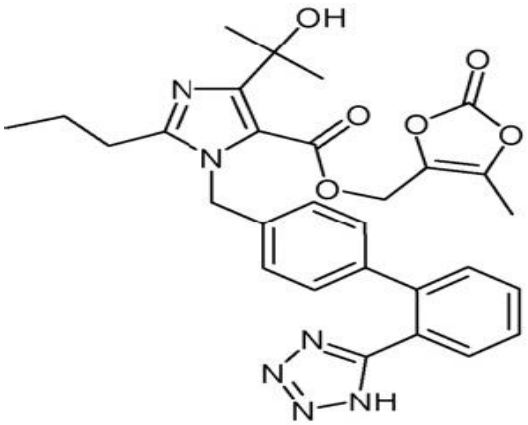

Figure 1. Structure of olmesartan medoxomil

2. Material and method

2.1. Chemical and reagent

The working standard of Olmesartan medoxomil was procured from Hetero drug Pvt. Ltd. India. HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt Germany). Deionised and ultrapure water was used in all experiments was obtained from Milli-Q system (Millipore). Ortho phosphoric acid used for adjusting the PH of buffer solution was AR grade (S. D .Fine chemicals).

2.2. Equipment

pH of the mobile phase was checked on a pH/ion analyser (Lab India, PHAN, India). Refluxing of drug in hydrolysis condition was carried out in round bottom flask-condenser assembly. The HPLC system employed in method development, forced degradation studies, and assay method validation was Shimadzu LC-8A pump, Shimadzu SCL-10AVP system controller and Shimadzu SPD-M10 AVP UV detector and Class VP software as data integrator.

2.3.1. Preparation of mobile phase

400 ml of Milli Q water, adjusted to pH 3.70 with 10 mM O-phosphoric Acid was mixed with 600 ml of Methanol. The final volume of 1000 ml resulting in pH 3.75 for final mobile phase. The mobile phase was sonicated for 20 min.

2.3.2. Preparation standard solution

Stock solution of Olmesartan Medoxomil 1 mg mL^{-1} was prepared in methanol. Standard solutions were prepared by dilution of stock solution with mobile phase to give solution in concentration of 0.060 to 10.000 µg mL^{-1}. The stock solution used for the degradation studies was 10 µg mL^{-1}.

2.3.3. Optimized chromatographic condition

The chromatographic separation was achieved on Qualisil BDS RP-C_{18} column (250×4.6 mm, 5 µm particle size) using a mobile phase consisting of mixture of methanol: 10 mM *o*-phosphoric acid (60:40, v/v), pH 3.75. All reagents were filtered through the 0.45 µm filter paper and sonicated before use. The injection volume was 100 µL. the Uv-Vis detector was set at the wavelength 270 nm. The assay was performed at 25^0C and the flow rate was fixed at 1 mg mL^{-1}.

2.3.4. Validation of method

The stock solution of the drug was prepared at strength of 1 mg mL^{-1}. It was diluted to prepare solutions containing 0.060 to 10.000 µg $mL^{-1}$of the drug Olmesartan medoxomil. The solution was injected in triplicates into a HPLC column keeping the injection volume constant (100 µL.)

Twelve injections of three different concentration [LQC (0.500µg mL^{-1}), MQC (5.000 µg mL^{-1}), HQC (7.500 µg mL^{-1})], were given at the same day and the values of relative standard deviation were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

Accuracy was calculated for the known concentration of the drug. The recovery of the added drug was determined. The method is specific as it is well resolved and distinguished from the degradation products.

The LOD and LOQ were determined at a signal to noise ratio of 3:1 and 10:1 respectively by injecting the dilute solution with known concentrations.

Robustness of method was investigated by varying the chromatographic condition such as change of flow rate (± 10%), organic content in mobile in phase (±2%), wavelength of detection (±5%), and the pH of buffer in the mobile phase (±0.2%). Robustness of the developed method was indicated by the overall %RSD between the data at each variable condition.

The solution stability was carried out by leaving both test solution of sample and reference standard in tightly capped volumetric flask at -20^0 C for 7 days. The sample solution was assayed after 7 days with fresh sample.

2.3.5. Stress degradation studies

Acid induced stress degradation was performed by adding 10 mL of stock solution (1mg mL^{-1}) of Olmesartan Medoxomil to 10 mL each of methanol and 0.1 M HCl and refluxing the mixture at 60^0 C for approximately for 60 min. The solution was left to reach ambient temperature. From this solution 1 ml was sufficiently diluted to 100 ml of methanol to get final concentration of 10 µg ml^{-1}.

Base induced stress degradation was performed by adding 10 mL of stock solution (1 mg mL^{-1}) of Olmesartan medoxomil to 10 ml of methanol and 0.1M NaOH and refluxing the mixture at 60^0C for approximately 60 min. The solution was then left to reach the ambient temperature.

From this solution 1 ml was sufficiently diluted to 100 ml of methanol to get final concentration of 10 μg ml^{-1}.

2.3.6. Oxidative degradation

To study the effect of oxidizing conditions, 10 mL of stock solution (1mg mL^{-1}) of Olmesartan Medoxomil was added to 10 mL of 30% H_2O_2solution and the mixture was refluxed at 60^0C for 60 min. The solution was then left to reach the ambient temperature. From this solution 1 ml was sufficiently diluted to 100 ml of methanol to get final concentration of 10 μg ml^{-1}.

2.3.7. Thermal degradation

To study the effect of temperature, approximately 100mg of olmesartan medoxomil was stored at 100^0C for 24 hour then it was dissolved in 10 mL of methanol and volume was adjusted up to 100 ml with mobile phase. From this solution 1 ml was sufficiently diluted to 100 ml of mobile phase to get final concentration of 10 μg ml^{-1} of Olmesartan Medoxomil.

2.3.8. Photolysis

To study the effect of UV light, approximately 100 mg of Olmesartan medoxomil was exposed to UV fluorescent lamp having spectral distribution from 320 to nm to 400 nm with a maximum energy emission between 350 nm to 370nm for 7 days then it was dissolved in 10 mL of methanol and volume was adjusted up to 100 ml with mobile phase. From this solution 1 ml was sufficiently diluted to 100 ml of mobile phase to get final concentration of 10 μg ml^{-1} of Olmesartan Medoxomil. 100 μL of resulting solution was injected into HPLC and chromatograms were recorded. The stability samples were recorded using UV detector, as the method was found to be rugged in nature.

3. Result and discussion

3.1. Degradation behavior

HPLC studies on olmesartan medoxomil under different stress condition suggested the following degradation behaviour (Table 1).

The % degradation was calculated by following formula:

% degradation = [(actual initial area of untreated stock solution – reduced area of treated stock solution)/actual initial area of untreated stock solution]*100.

The rate of hydrolysis in acid and alkali was fast and significant reduction in peak area, with degradation product was observed in sample.

It was observed that on heating at 60 ^{0}C in 0.1 M HCl height of drug decreased with respect to time in solution, with an additional peak at 4.56 min, 5.98min, 6.21 min, this indicate drug is hydrolysed under strong acidic condition. Olmesartan medoxomil found to be degraded 47.56 % in acidic conditions. (Figure 3)

The drug was found to be highly susceptible to alkaline hydrolysis. The reaction in 0.1 N NaOH at 60 ^{0}Cwas so rapid that 48.92% of the drug was degraded in 60 min time forming degradation product at 4.89 min , 6.38 min , 9.84 min ,11.87, 14.67 min. (Figure 4)

The drug was found to be highly susceptible to oxidation with 3% hydrogen peroxide at 50^0C

temperature. It was decomposed to 41.88 %. Major degradation products were observed at Rt 3.01 min, 5.09 min, 1.91 min, 14.71 min, 15.89 min, which indicated that the drug is degraded in oxidative conditions. (Figure 5)

Olmesartan medoxomil also proved labile to thermal degradation after treating drug to 100 ^{0}C. Minor degradations were observed at Rt 4.85 min 9.96 min, 11.86 min, 12.33 min, after exposure of drug for 24 Hrs, which indicate that drug was unstable under thermal condition in solid form. Olmesartan medoxomil was found to be degraded 26.38 %. (Figure 6)

In comparison to acid and alkali hydrolysis the drug was reasonably stable to photolysis where no degradation was found when olmesartan exposed to UV radiation for 7 days. (Figure 7).

Table 1. Percent degradation of olmesartan medoxomil and retention time of degradation products

Sr. No.	Degradation condition	Retention time of degradation products (Min)	Peak area (µV.sec)	Mass Concentration (µg mL-1)	Percent Degradation of drug (n = 5)
1	Untreated stock solution (10µg mL-1)	7.92	3681856.50	10	------
2	Acid Hydrolysis	2.21 5.98 6.21 7.92	1005042.00 702221.25 111121.75 1864492.50	4.75	47.56%
3	Base Hydrolysis	4.89 6.38 7.90 9.84 11.87 14.67	631021.00 90102.25 1733418.50 911003.50 201301.50 115001.00	4.89	48.92%
4	Oxidation	3.01 5.09 7.91 11.91 14.71 15.89	298241.00 253341.50 2213534.50 274734.25 121421.75 283717.50	4.18	41.88%
5	Thermal degradation	4.85 7.92 9.96 11.86 12.23	219241.25 26946776.50 254491.50 89080.00 224395.75	2.63	26.38%
6	Photolytic degradation	7.92	3681915.50	--------	--------

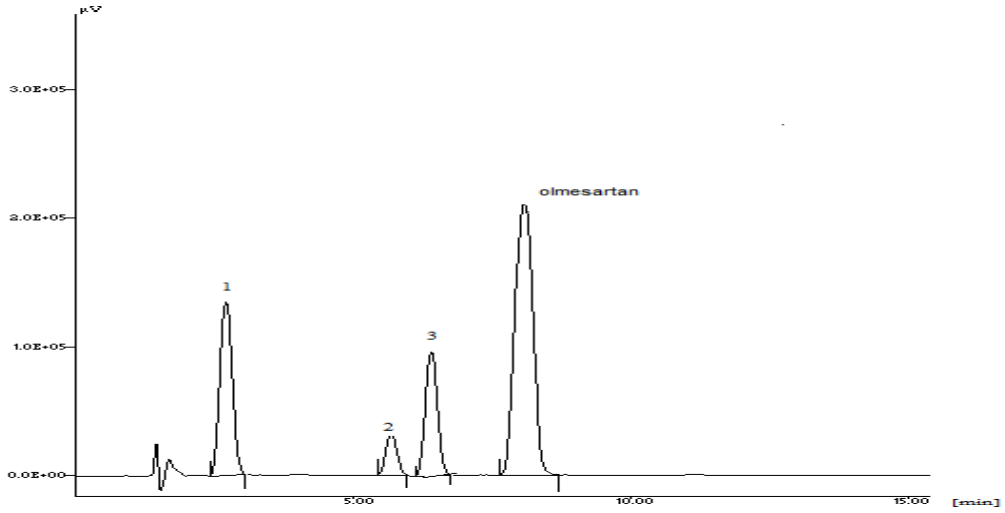

Figure 3. Chromatogram of olmesartan medoxomil degraded with acid hydrolysis

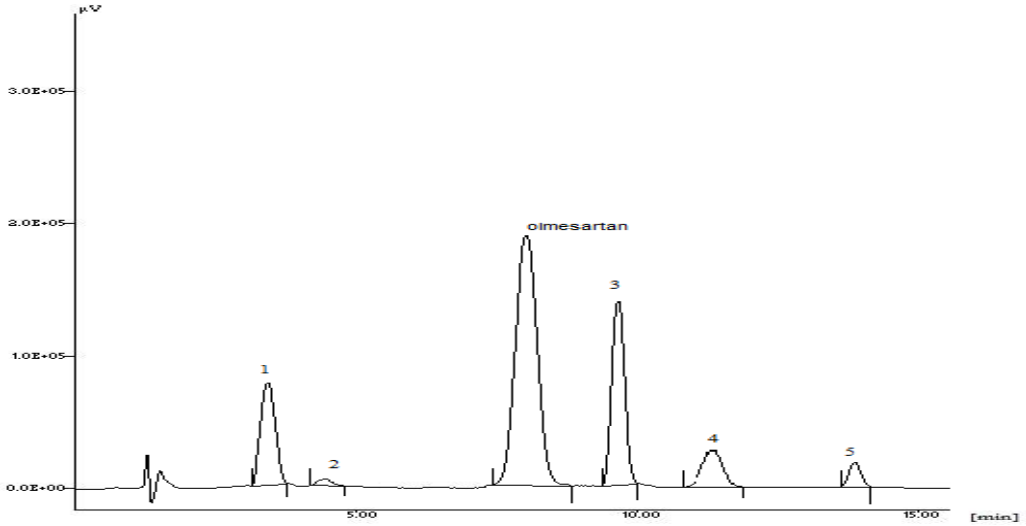

Figure 4. Chromatogram of olmesartan medoxomil degraded under alkaline conditions

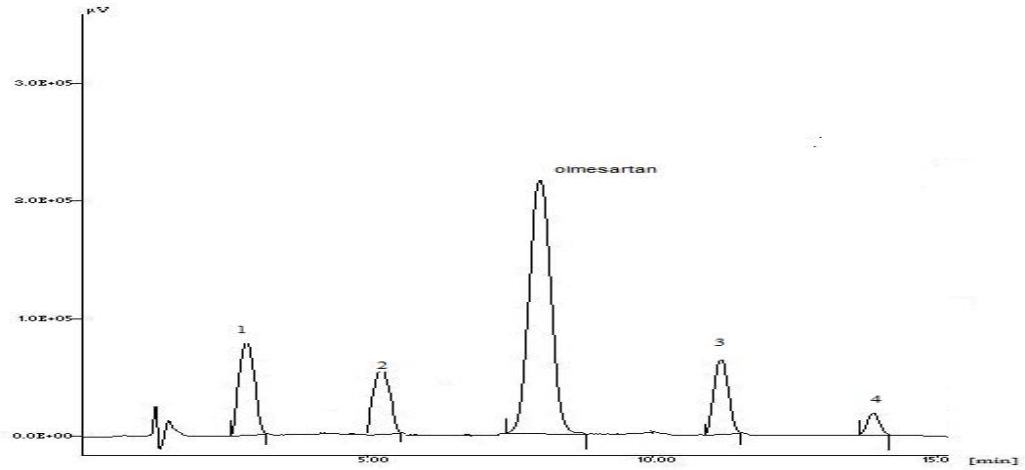

Figure 5. Chromatogram of olmesartan medoxomil after oxidation

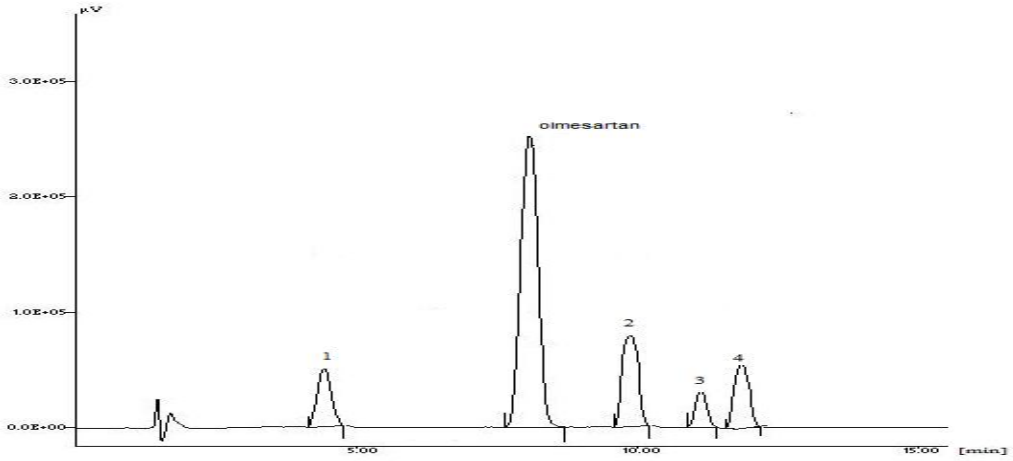

Figure 6. Chromatogram of olmesartan medoxomil after thermal degradation

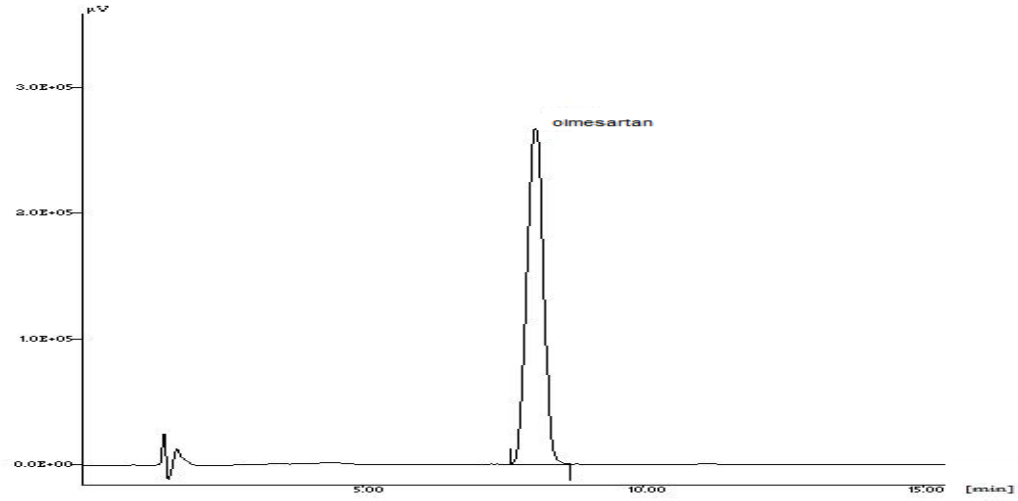

Figure 7. Chromatogram of olmesartan medoxomil after photolytic degradation

3.2. Establishment of stability-indicating method

Olmesartan medoxomil is weak acidic in nature (ionised in alkaline medium) so reversed phase chromatography was considered as the best choice. Separation of olmesartan from its degradation product has been performed on C18 column. The mobile phase was optimised with different ratio of O-phosphoric acid as buffer and methanol. The proportion of methanol in the mobile phase was alter to get good resolution and desired retention time. Increasing the methanol ratio was accompanied by decreased in retention time of different component; however the separation was still achieved. Since pKa of olmesartan is 13.0 so in the acidic pH probability of drug being in ionised form is more, which in turn has an effect on peak shape and retention time. This statement supported when improved peak shape, tremendous decrease in tailing and reproducible response observed between pH ranges of 3.5 to 5.0. In order to ensure complete separation and high resolution, the chosen ratio was methanol: 10mM O-phosphoric acid (pH 3.70)-(60:40%v/v) as a mobile phase. Final pH of the mobile phase was 3.75.

Olmesartan medoxomil showed maximum wavelength at 270 nm. The specificity of the method is illustrated in Figure 2 and the average retention time of Olmesartan medoxomil for 10

replicates was 7.92± 0.05 minutes. Construction of calibration curve was performed by transferring the aliquots of olmesartan stock and working standard solution into a series of 10 mL volumetric flask and diluting to volume with mobile phase to obtain solution in concentration range of 0.06 µg ml^{-1} to 10.0 µg ml^{-1}. 50 µL volumes from each solution were injected in multiple of 5 injections. Chromatographic separation was run under previously mentioned conditions. All determination was performed at ambient temperature. The average peak area obtained for each concentration was plotted versus concentration.

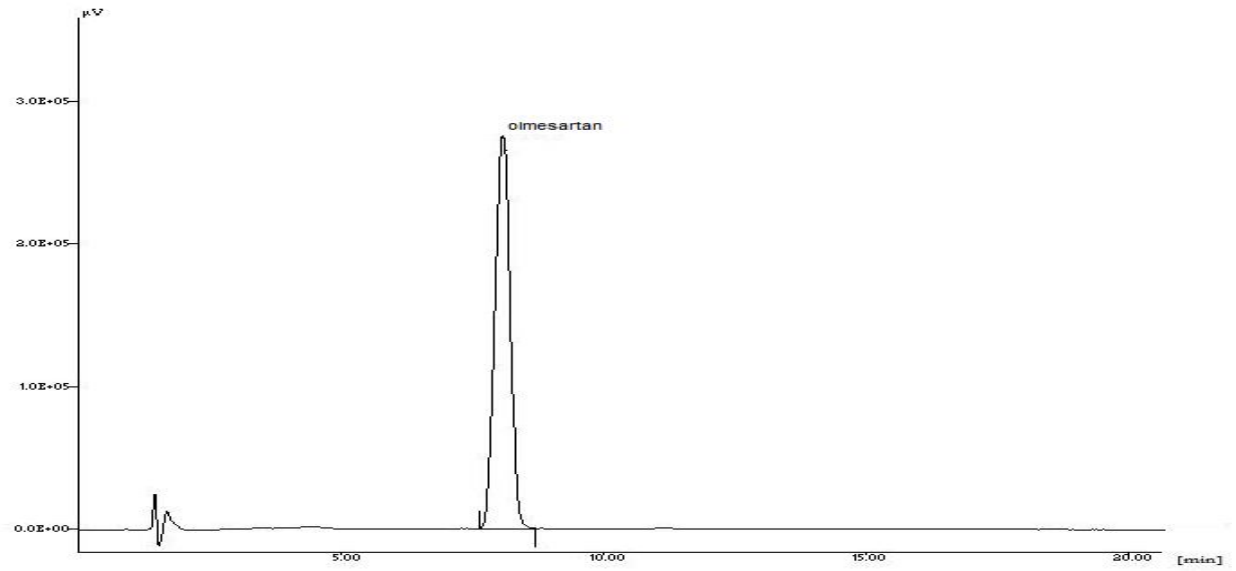

Figure 2. Chromatogram of working standard olmesartan medoxomil

4. Validation of method

The method was validated with respect to linearity, precision, accuracy, specificity and robustness. The response for the drug was linear in the studied concentration range (r^2 = 0.9981). The mean (±R.S.D.) values of slope and correlation coefficient were 375863 (±3224.94) and 0.9981 (±0.001), respectively. (Table 2).

Table 3 provides data obtained from the precision experiments. The R.S.D. values for intra- and inter-day precision were < 2 %, thereby indicating that the method was sufficiently precise. The method was found to be specific to the drug. The drug peak was free from any co eluting peak. The result indicated that the method was highly precise. Good separations were always achieved which suggested that the method was selective for all components under the test. The LOD and LOQ concentrations were found to be 0.020 μg mL^{-1} and 0.060 μg mL^{-1}.Influence of small changes in chromatographic conditions such as change in flow rate (±10 %), organic content in mobile phase (±2 %), wavelength of detection (±5 %) and pH of buffer in mobile phase (±0.2 %) studied to determine the robustness of the method are also in favor (% R.S.D. <2 %) of the developed HPLC method for the analysis of Olmesartan Medoxomil. The % R.S.D. of the assay of Olmesartan medoxomil during solution stability experiments were within 2%. No significant changes were observed during solution stability. The solution stability data confirms that the sample solutions were stable at least for 7 days.

Table 2. Linearity and range

Linearity and range	Olmesartan medoxomil
Range (µg mL-1)	0.06 to 10
r2	0.9981
Slope	375863
Intercept	29656

Table 3. Precision and recovery data

Precision			**% Recovery**	
Actual Concentration (µg/mL)	**Measured Concentration (µg/mL) ± S.D.; % R.S.D.**			
	Intra-day	Inter-day	Intra-day	Inter-day
0.500	0.502±0.002;2.37	0.501±0.005;2.29	100.41	100.33
5.000	5.012±0.013;1.95	5.029±0.083;2.63	100.25	100.58
7.500	7.539±0.029;1.47	7.504±0.042;1.93	100.52	100.06

5. Conclusion

The study shows that the developed HPLC Method is fast, precise, specific, accurate and stability indicating. The stability-indicating method resolved the drug peak and also the peaks of degradation products formed under variety of conditions. After exposure of Olmesartan medoxomil to stress conditions, it was concluded that the drug is susceptible to acid, base hydrolysis; oxidation, thermal degradation and photolysis with maximum degradation observed in base hydrolysis followed by thermal degradation. Therefore this method can be employed for monitoring the stability of Olmesartan medoxomil drug substance commercially.

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References

[1] Chrysant, S. G., and Chrysant, G. S. 2004. Antihypertensive efficacy of olmesartan medoxomil alone and in combination with hydrochlorothiazide. *Expert Opinion on Pharmacotherapautics,* 37: 657.

[2] Chrysant, S. G., Weber, M. A., Wang, A. C., and Hinman, D. J. 2004. Evaluation of antihypertensive therapy with the combination of olmesartan medoxomil and

hydrochlorothiazide. *American Journal of Hypertension,* 17: 252.
[3] Dongyang, L., Pei, H., Nobuka, M., Xiaoming, L., Li, L., and Ji, J. 2007. Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. *Journal of Chromatography B,* 856: 190-197.
[4] Ewing, G. W. 1995. *Instrumental Methods of Chemical Analysis,* 5th edn. Lippincott - Raven, Philadelphia.
[5] ICH. 2003. Stability Testing of new drug substances and products Q1A (R2): International Conference on Harmonization, IFPMA, Geneva, Switzerland. International Conference on Harmonization Draft guidelines on validation of analytical procedures: definitions and terminology, Federal Register, 1995. 60, Switzerland, 11260 28.
[6] Laeis, P., Puchler, K., and Kirch, W. 2001. The pharmacokinetic and metabolic profile of olmesartan medoxomil limits the risk of clinically relevant drug interaction. *Journal of Hypertension,* 19: 21-32.
[7] Laeis, P., Puchler, K., and Kirch, W. 2001. *Journal of Hypertension,* 19, (Suppl 1): S21-S32 Lui, D., Hu, P., Matsushima, N., Li, X., Li, L., Jiang, J. 2007.
[8] Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. *Journal of Chromatography B. Analytical Technology in Biomedical Life Science,* 856: 190-197.
[9] Murakami, T., Konno, H., Fukutsu, N., Onodera, M., Kawasaki, T., and Kusu, F. 2008. Identification of a degradation product in stressed tablets of olmesartan medoxomil by the complementary use of HPLC Hyphenated Techniques. *Journal of Pharmaceutical and Biomedical Analysis*, 47: 553-559.
[10] Mustafa, C. and Sacide, A. 2007. Development of a CZE Method for the determination of olmesartan Medoxomil in tablets. *Chromatography,* 66: 929-933.
[11] Nakamura, H., Inoue, T., Arakawa, N., Shimuzi, Y., Yoshigae, Y., Fujimori, I., E, Shimakawa., T, Toyoshi., and T, Yokoyama. 2005. Pharmacological and pharmacokinetic study of olmesartan medoxomil in animal diabetic retinopathy models. *Europian Journal of Pharmacology,* 512: 239-246.
[12] Notari, S., Bocedi, A., Ippolito, G., Narciso, P., Pucillo, L. P., Tossini, G., Donnorso, R. P., Gasparrini, F., and Ascenzi, P. 2006. Simultaneous determination of olmesartan medoxomil in human plasma by high-performance liquid chromatography. *Journal of Chromatography B Biomedical Applications,* 831: 258-266.
[13] Settle F. 1997 *"Handbook Of Instrumental Techniques For Analytical Chemistry"*. Prentice Hall inc 17-18, 247-277, 309-334, 567 -582.
[14] Miller, J. M. and Crowther, J. B. 2000. Analytical Chemistry In GMP Environment - A Practical Guide, John Wiley and Sons Page no.279-281, 350-351.
[15] Eriksson, L., Johansson, E., Kettaneh-Wold, N., and Wold, S. 2001. *"Multi - and Megavariate Data Analysis, Principles and Applications."* 1st Edition, Umetrics Academy, June 07, 3-4.
[16] Yamasaki Y., Katakami N., Kutakami R., and Hayaishi-Okano. 2005. HPLC analysis of olmesartan in blood plasma, Diabetes Research and Clinical Practice, 67: 204-210.
[17] Miroshnichenko, I. I. and Yurchenko, N. I. 2002. HPLC Analysis for Omeprazole and Lansoprazole in Blood Plasma. Translated from *Khimiko-Farmatsevticheskii Zhurnal,* 36, 7: 48-49.
[18] Skoug, J. W. 1996. Strategy for development and validation of dissolution test for solid Dosage Forms. *Pharm Tech*, 20, 5: 58-60
[19] Brewer E., and Henion J. D. 1998. Atmospheric pressure ionization LC/MS/MS techniques

for drug disposition studies. *Journal of Pharmaceutical Science*, 87: 395-402.

[20] Wilson, I. D., Griffiths, L., Lindon, J. C., and Nicholson, J. K. 2000. in: S. Gorog (Ed.), *"Identification and Determination of Impurities in Drugs"*. Elsevier, Amsterdam, 299-322.

[21] Lindon, J. C., Nicholson, J. K., and Wilson, I. D. 2002. in: K. Albert (Ed.), *"On-line LC - NMR and Related Techniques"*. Wiley, Chichester, 45-87.

[22] Murakami, T., Fukutsu, N., Kondo, J., Kawasaki, T., and Kusu F. 2008. Application of liquid chromatography-two-dimensional nuclear magnetic resonance spectroscopy using pre-concentration column trapping and liquid chromatography-mass spectrometry for the identification of degradation products in stressed commercial amlodipine maleate tablets. *Journal of Chromatography A,* 1181, 67-76.

[23] Fukutsu, N., Kawasaki, T., Saito, K., and Nakazawa, H. 2006. Application of high-performance liquid chromatography hyphenated techniques for identification of degradation products of cefpodoxime proxetil. *Journal of Chromatography A,* 1129: 153-159.

[24] Somsen, G. W., Gooijer, C., Velthorst, N. H., and Brinkman, U. A. Th. 1998. Coupling of column liquid chromatography and Fourier transform infrared spectrometry. *Journal Chromatography A.* 811: 1-34.

[25] Somsen, G. W., Gooijer, C., and Brinkman, U. A. Th. 1999. *Journal of Chromatography.A 856,* 213-242.

[26] Wilson, I. D., and Brinkman, U. A. Th. 2007. Hype and hypernation: multiple hyphenation of column liquid chromatography and spectroscopy. *Trends Analytical Chemistry,* 26: 847-854.

[27] Mizuno, M., Sada, T., Ikeda, M., Fukuda, N., Miyamoto, M., Yanagisawa, H., and Koike, H. 1995. Pharmacology of CS-866, A Novel Nonpeptide Angiotensin-II Receptor Antagonist. *Europian Journal Pharmacology.* 285: 181-188.

[28] Takemoto, M., Egashira, K., Tomita, H., Usui, M., Okamoto, H., Kitabatake, A., Shimokawa, H., Sueishi, K., and Takeshita, A. 1997. Chronic angiotensin-converting enzyme inhibition and angiotendin II type 1 receptor blockade. *Journal of Hypertension,* 30: 1621-1627.

[29] Puchler, K., Nussberger, J., Laeis, P., Witte, P. U., and Brunner, H. R. 1997. Blood pressure and endocrine effects of single doses of CS-866, a novel angiotensin II antagonist, in salt-restricted hypertensive patients. *Journal Hypertension,* 15: 1809-1812.

[30] Zhao, Z., Wang, Q., Tsai, E. W., Qin, X. Z., and Ip, D. 1999. Identification of losartan degradates in stressed tablets by LC-MS and LC-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis,* 20: 129-136.

[31] Puchler, K., Laeis, E., and Gunther, A. 1999. Safety, tolerability and efficacy of the new oral angiotensin II (AT1)-receptor antagonist CS-866 in patients with mild to moderate hypertension. *J. Hum. Hypertens,* 13:S4.

[32] Rane, V. P., Patil, K. R., Sangshetti, J. N., Yeole, R. D., and Shinde, D. B. 2009. Stability-indicating LC method for the determination of olmesartan in bulk drug and in pharmaceutical dosage form. *Chromatography,* 69: 169-173.

[33] Vaidya, V. V., Roy, S. M. N., Yetal, S. M., Joshi, S. S., and Parekh, S. A. LC-MS-MS determination of olmesartan in human plasma. *Journal of mass Chromatography,* 67:147-150.

[34] Zhao, Z., Wang, Q., Tsai, E. W., Qin, X. Z., and Ip, D. 1999. Identification of losartan degradates in stressed tablets by LC-MS and LC-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis*, 20:1.