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Method Development and Validation for Simultaneous Estimation of *Olmesartan Medoxomil* and *Hydrochlorothiazide* by RP-HPLC

SURYADEVARA VIDYADHARA*, REDDYVALAM LANKAPALLI CH SASIDHAR, BALLIPALLI VENKATESWARA RAO, KODURI TEJASWI and MARUPUDI RESHMA

Chebrolu Hanumaiah Institute of Pharmaceutical Sciences,
Chandramoulipuram, Chowdavaram, Guntur - 522 019, India.
*Corresponding author E-mail: svidyadhara@gmail.com

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ABSTRACT

A simple, fast, precise reverse phase isocratic high performance liquid chromatographic (HPLC) method has been developed for the simultaneous estimation of Olmesartan medoxomil (OM) and hydrochlorothiazide (HCTZ) in marketed formulations. Estimation of drugs in this combination was done with a C18 column [ODS UG 5 column, 250mm x 4.5mm] using mobile phase of composition acetonitrile and phosphate buffer (50:50 v/v, pH 6.8). The flow rate was 1.0 ml/min and the separation was monitored at 260nm. The retention time of HCTZ and OM were 3.6min and 4.3min respectively. The method was found to be linear over a range of 10-70µg/ml for OM and 6-42µg/ml for HCTZ. The method was validated according to the guidelines of International Conference on Harmonisation (ICH) and was successfully employed in the estimation of commercial formulations.

Key words: Olmesartan medoxomil, Hydrochlorothiazide, RP-HPLC, Method validation.

INTRODUCTION

Olmesartan medoxomil(OM) is the latest angiotensin II receptor blocker approved for use as an alternative therapeutic anti-hypertensive agent. Chemically, OM is described as (5- methyl-2- oxo-1, 3- dioxol-4-yl) methyl ester of 4-(1- hydroxy-1-methylethyl)-2-propyl-1-{[22 -(1Htetrazol- 5-yl) [1, 12 -biphenyl]-4-yl] methyl}-1Himidazole- 5-carboxylic acid[1]. Hydrochlorothiazide is one of the oldest and widely used diuretics which is also used in the treatment of hyper-tension[2-3]. Chemically, it is 6-chloro- 3, 4-dihydro- 2H- 1, 2, 4-benzothiadiazine-7-sulphonamide 1, 1- dioxide.

Several analytical methods have been reported for the estimation of HCTZ in marketed formulations and United States Pharmacopoeia (USP) describes a RP-HPLC method for its estimation [4-12].OM has not been described officially in any pharmacopoeia yet. Extensive literature survey revealed that very few methods were reported for the simultaneous estimation of OM and HCTZ. So, an attempt has been made to develop an accurate, precise and economically viable RP-HPLC method for the simultaneous estimation of combination of interest in the current research.

MATERIALS AND METHODS

Equipment used

The chromatographic separation was performed on Agilent 1120 compact Liquid Chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20 µl fixed loop. A reverse phase C18 column [Agilent ODS UG 5 column, 250mm x 4.5mm] was used. Elico SL 218 double beam UV-visible spectrophotometer and Axis AGN204–PO electronic balance were used for spectrophotometric determinations and weighing purposes respectively.

Reagents and chemicals

Pharmaceutical grade pure Olmesartan medoxomil and Hydrochlorothiazide were obtained as gift samples from Apotex Research Private Limited (ARPL), Bangalore and Life line Formulations, Vijayawada respectively. Marketed formulation Olsertain-H 20 with dose of 20mg of OM and 12.5mg of HCTZ were procured from local market. HPLC grade Acetonitrile and Water were procured from Merck specialities private limited, Mumbai.

Chromatographic conditions

C_{18} column [Agilent ODS UG 5 column, 250mm x 4.5mm] was used for the chromatographic separation at a detection wavelength of 260nm. Mobile phase of composition acetonitrile and phosphate buffer, pH 6.8 in a ratio of 50:50 was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 1ml/min and the injection volume was 20µL.

Preparation of mobile phase

Phosphate buffer was prepared by mixing 22.4ml of 0.2M sodium hydroxide to 50ml of 0.2M potassium dihydrogen ortho phosphate and the pH was adjusted to 6.8. 450ml of buffer was added to 450ml of acetonitrile, filtered through 0.45µ membrane filter and sonicated for 20 minutes.

Preparation of standard solutions

25mg each of OM and HCTZ were accurately weighed and transferred into two 25ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solutions A (OM) and B (HCTZ) of concentration 1000µg/ml of each drug. From the primary stock solutions, 10ml and 6ml were pipetted out from A and B respectively, transferred to a 50ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 200 and 120µg/ml of OM and HCTZ respectively (working stock solution A).

Preparation of sample solution

Twenty tablets of Olsertain-H 20 were weighed and crushed. Content equivalent to 50mg OM and 30mg HCTZ was weighed accurately and transferred to a 50ml volumetric flask. The content was dissolved with 20ml of solvent and then sonicated for 15min. The volume was made up with the mobile phase and filtered with 0.45µ membrane filter. 1ml from the primary stock solution was pipetted out and transferred to a 10ml volumetric flask and the volume was made up with the solvent to obtain a concentration of 100 and 60 µg/ml of OM and HCTZ respectively (working stock solution B).

Optimization of HPLC method

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of OM and HCTZ. For the method optimization, different mobile phases were tried but acceptable retention times, theoretical plates and good resolution were observed with acetonitrile and pH 6.8 phosphate buffer (50:50, v/v) using C_{18} column (Agilent ODS UG column, 250mm x 4.5mm)

Validation of the RP-HPLC method

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

System suitability

System suitability was carried out with five injections of solution of 100% concentration having 70µg/ml of OM and 42µg/ml of HCTZ in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T) were reported in table 1.

Linearity

For the determination of linearity, appropriate aliquots were pipetted out from working stock solution A to a series of 10ml volumetric flasks and the volume was made up with the solvent to obtain concentrations ranging from 10-70 µg/ml of OM and 6-42 µg/ml of HCTZ. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for OM and HCTZ were shown in Figure 3 and their corresponding linearity parameters were given in table 2.

Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percent recovery was reported. The results were given in Table 4.

Precision

The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (70µg/ml of OM and 42µg/ml of HCTZ) on the same day and for intermediate precision %RSD was calculated from repeated studies on different days. The results were given in table 3.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/S and LOQ = 10 σ/S. the values were given in table 2.

Robustness

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wavelength, etc. and the % RSD should be reported.

Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ±2nm in the detection wavelength and ±0.1 mL/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions were injected to the instrument in triplicate. %RSD was reported in table 5.

Assay of marketed formulation

20µl of sample solution of concentration 70µg/ml of OM and 42µg/ml of HCTZ was injected into chromatographic system and the peak responses were measured. The solution was injected three times into the column. The amount present in each tablet was calculated by comparing the areas of standards with the test samples.

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, acetonitrile and phosphate buffer, pH 6.8 50:50 v/v was selected as mobile phase because of better resolution and symmetrical peaks. OM and HCTZ were found to show appreciable absorbance at 260nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of HCTZ and OM at different R_Ts was shown in figure 2.

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatogram confirms the presence of HCTZ and OM at 3.6min and 4.3min respectively without any interferences. The parameters were given in table 1.

Table 1: System suitability parameters

Parameters	Hydrochlorothiazide	Olmesartan medoxomil
Retention time (min)	3.6	4.3
Theoretical plates (N)	11421	9897
Tailing factor (T)	1.2	1.4
Resolution (R_s)		1.9

Table 2: Results for Linearity (n=3)

Parameters	Hydrochlorothiazide	Olmesartan medoxomil
Slope	963140	727006
Intercept	586967	17530
r^2	0.9996	0.9996
Regression equation	y = 963140x + 586967	y = 727006x + 17530
Linearity range	6-42µg/ml	10-70 µg/ml
LOD	0.10 µg/ml	0.25 µg/ml
LOQ	0.32 µg/ml	0.78 µg/ml

*n= No. of determinants

Table 3: Results for Precision (n=6)

Drug	Intraday Precision (% RSD)	Interday Precision (% RSD)
Hydrochlorothiazide	0.69	1.13
Olmesartan medoxomil	0.57	0.72

*n= No. of determinants

Table 4: Results for Accuracy (n=3)

Recovery level	Olmesartan medoxomil				Hydrochlorothiazide			
	Amount Added (µg/mL)		Amount Recovery	% Found (µg/mL)	Amount Added (µg/mL)		Amount Found (µg/mL)	% Recovery (µg/mL)
	Std.	test			Std.	test		
80%	40	10	49.51	99.02	24	6	30.28	100.94
100%	50	10	60.93	101.55	30	6	36.21	100.59
120%	60	10	69.61	99.44	36	6	41.32	98.39
Mean Recovery	99.02-101.55% w/w				98.39-100.94%w/w			

*n= No. of determinants

Table 5: Results for Robustness

Parameters (n=3)	% RSD	
	Hydrochlorothiazide	Olmesartan medoxomil
At λ max 258 nm	0.42	0.43
At λ max 262 nm	0.71	0.59
With flow rate 0.9 ml	0.66	0.48
With flow rate 1.1 ml	0.72	0.78

*n= No. of determinants

Table 6: Results for Assay (n=3) of marketed formulation

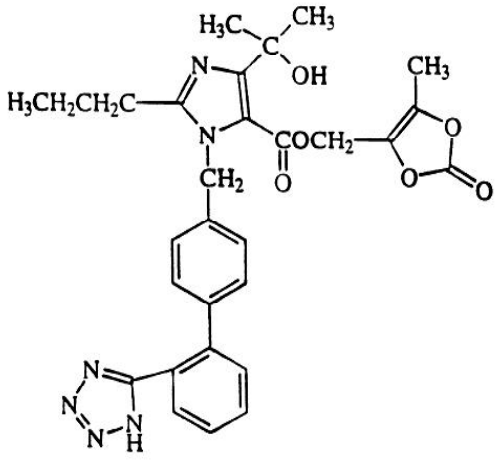
Drug	Label claim (mg/tab)	Amount recovered	% Amount found in drug
OM	20 mg	20.06 mg	100.34 % w/w
HCTZ	12.5 mg	12.4 mg	99.2%w/w

*n= No. of determinants

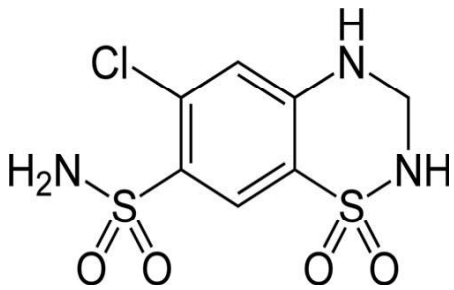
Concentration range of 10-70 µg/ml for OM and 6-42µg/ml for HCTZ were found to be linear with correlation coefficients 0.9996 and 0.9996 for OM and HCTZ respectively. The results were given in table 2.

The proposed method was found to be precise and reproducible with %RSD of 1.13 and 0.72 for HCTZ and OM respectively. %RSD was reported in table 3.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 98.39 to 100.94% w/w and 99.02-101.55%ww/w for HCTZ and OM respectively which indicates that the method was accurate. Values obtained were given in table 4.



a) Olmesartan medoxomil



b) Hydrochlorothiazide

Fig. 1: Chemical Structures of a) OM and b)HCTZ

The limits of detection for HCTZ and OM were found to be 0.106µg/ml and 0.259 µg/ml respectively and the limits of quantitation were 0.323 µg/ml and 0.786 µg/ml respectively.

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well

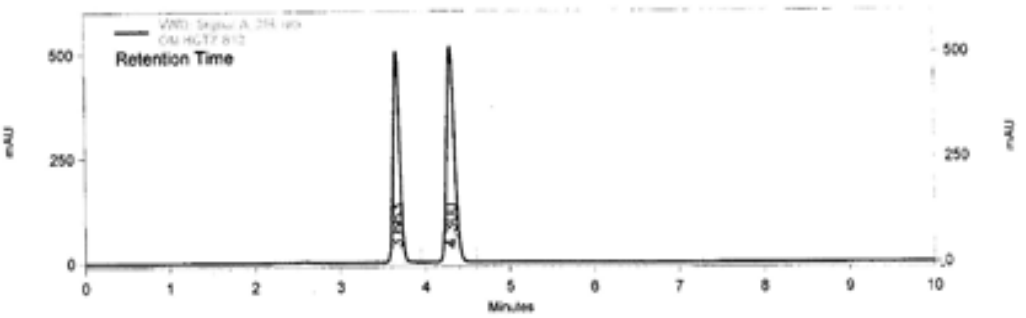


Fig. 2: Optimized chromatogram of HCTZ and OM by RP-HPLC

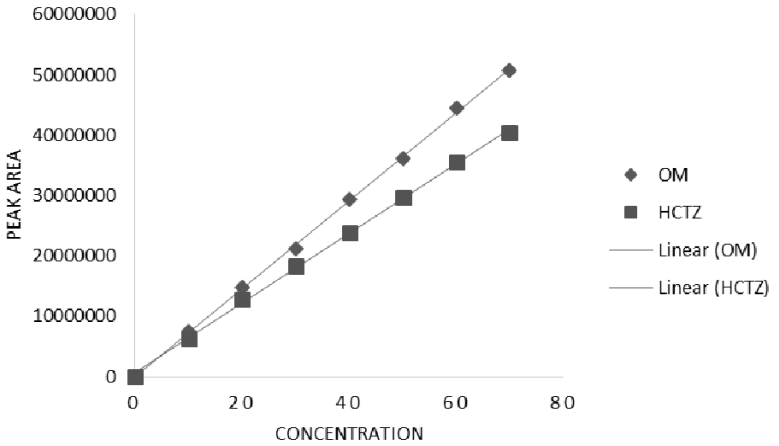


Fig. 3: Calibration Plot of Olmesartan medoxomil and Hydrochlorothiazide

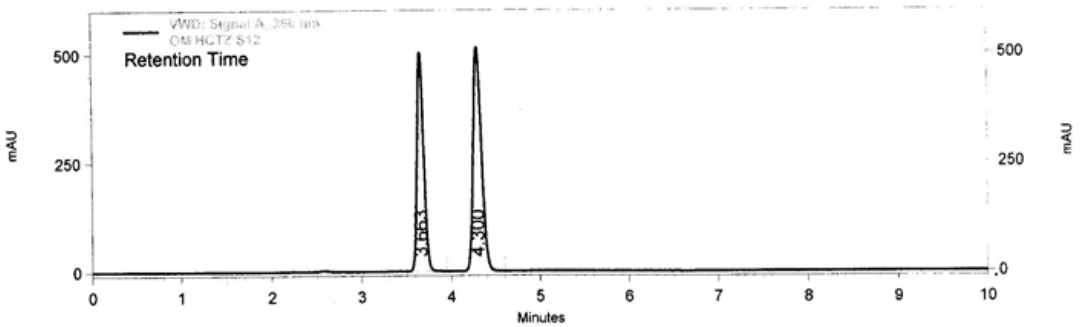


Fig. 4: A typical chromatogram for assay of marketed formulation containing 42 µg/ml of HCTZ and 70 µg/ml of OM

suitable for the estimation of the commercial formulations of the selected combination. Values obtained were given in table 5.

The method was found to be robust after changing the conditions like detection wavelength (± 2nm) and flow rate (± 0.1 ml). %RSD was calculated for each variation and reported. Values obtained were given in table 6.

CONCLUSION

The HPLC method developed and validated allows a simple and fast quantitative determination of Olmesartan medoxomil and Hydrochlorothiazide from its formulation. All the validation parameters were found to be within the limits according to the ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged, robust and can be involved in the routein analysis of the marketed formulations.

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REFERENCES

1. Connick R. E. and Hugus Z. Z., *J. Am. Chem. Soc.,* **75**: 6012 (1988)
2. Hans R.B. *Vasc Health Risk Manag.*, **2**: 327–340 (2006).
3. G.S. Devika, M. Sudhakar and J. Venkateshwar Rao, *Orient J. Chem.,* **28**(2): 887-893 (2012).
4. Beermann B, Groschinsky-Grind M and Rosen A. *Clin Pharmacol Ther.*, **19:** 531–7 (1976)
5. Raja B and Lakshmana Rao A. *Int J Res Pharm Chem.,* **03**: 714-717 (2011)
6. Raveendra G.B, Ramprasad LA, Srinivasu P, Jayachandra PR and Mustafa M. Eurasian *J. Anal. Chem.,* **5**: 145-151 (2009)
7. Parthiban C, Bhagavan Raju M and Sudhakar M. *Int J Pharm Indus Res.,* **1**: 325-329 (2011).
8. F. Shokraneh, A. Dabirsiaghi and N. Adib, *Orient J. Chem.*, **28**(1): 237-241 (2012).
9. Della Grace T.P, Molly M, Ganesan V, Anila J and Revikumar K.G. *Int Jour Pharm Sci Rev Res.,* **4**: 36-39 (2010).
10. Vachhani K.H and Patel Satish A. *Journal of Applied Pharmaceutical Science.*, **1**: 112-115 (2011).
11. Chouhan K.S, Bhure M.V, Hemke A.T, Gupta K.R, Wadodkar S.G. *Res. J. Pharm. Biol. Chem. Sci.*, **1**: 78-84 (2010).
12. Hasan M, Al Masud A, Ahmed J. *International journal of Pharmaceutical sciences and research.*, **1**: 80-84. (2010).
13. Maher K, Numan M, Murad A, Raqi S, Nidal *J. Int J Pharm Pharm Sci.,* **4**: 683-687 (2012).