ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development of Rp-Hplc Method For Estimation Of Hydrochlorothiazide and Olmesartan Medoxomil in Pharmaceutical Formulation

Chouhan KS, Bhure MV, Hemke AT, Gupta KR* and Wadodkar SG

Department of Pharmaceutical Chemistry, SKB College of Pharmacy, New Kamptee-441002, Nagpur (MS)

ABSTRACT

A simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous estimation of Hydrochlorothiazide and Olmesartan medoxomil in combined dosage form by RP-HPLC method. RP-HPLC estimation of drugs in selected combination was done using Phenomenex ODS 5µ, C_{18} column (250×4.6mm) and ACN: Methanol: Buffer (20:20:60) as mobile phase which shows sharp and resolved peak when detected at 230nm. The linearity range was found to be in concentration range 20-100 µg/mL for Hydrochlorothiazide (HTZ) and 32-160 µg/mL for Olmesartan medoxomil (OLM). The retention time for Hydrochlorothiazide and Olmesartan were 6.503 and 8.613 respectively. The correlation coefficient was found to be 0.9989 (for HTZ) and 0.9992 (for OLM). The mean percentage recovery was found to be 100.62 and 99.42 for HTZ and OLM respectively. The % estimation of the drugs was found near to 100 % representing the accuracy of the method. Validation of the proposed method was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed method can be successfully applied in routine work for the determination of Hydrochlorothiazide and Olmesartan medoxomil in combined dosage form.

Keywords: Hydrochlorothiazide, Olmesartan medoxomil, RP-HPLC method, tablets

*Corresponding author
Email ID: krishnargupta@rediffmail.com
Tel: +91-7109-288650

ISSN: 0975-8585

INTRODUCTION

Hydrochlorothiazide (HTZ) is a drug used in the treatment of hypertension. It blocks the reabsorption of Na+ in the distal convoluted tubules by inhibiting the luminal membrane bound Na+ / Cl- cotransport system. HTZ chemically [1], is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide1, 1 dioxide. It is a diuretic and highly soluble in methanol. Olmesartan medoxomil (OLM) is angiotensin II receptor antagonists used in the treatment of hypertension. OLM chemically [2] it is 2,3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl) -2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl] imidazole-5-corboxylate, cyclic 2,3-corbonate. Literature survey revealed that the methods reported earlier were only for the analysis of single drug like UV-spectrophotometry [3] for HTZ and LC [4], UV [5] and Capillary electrophoresis [6] for determination of OLM. In combination methods are reported for HTZ with Telmisartan [1], Irbesartan [8], Valsartan [9] and Lisinopril [10] while OLM with Ramipril [11] for their determinations in pharmaceutical formulation. HPTLC [12] method is reported for the HTZ with OLM combination. This paper presents simple, accurate, reproducible, rapid HPLC method for simultaneous analysis of the two components in tablet formulation.

MATERIALS AND METHODS

Instrument

Shimadzu HPLC 1100 series chromatograph equipped with binary pump LC-10 ADvp, SPD-10 UV detector, Rheodyne Manual injector 7725i with 20µL loop and a reversed phase 5µ Phenomenex OSD C18 column (250 x 4.6 mm) with pore size of 100A^{o} was used for the chromatographic studies. Shimadzu AUX220 balance was used for weighing the samples. All the chemicals used were of HPLC grade. Double distilled water and Whatmann filter paper (no.41) were used throughout the experimental work.

Materials

Multi-component tablet Olmetor-H (HTZ 12.5mg and OLM 20.0mg) manufactured by Hetero drugs ltd, Dist- Baddi, Himachal Pradesh. All chemicals and reagents used were of HPLC grade.

Mix Standard solution

Mix Stock solution containing HTZ and OLM was prepared in methanol having concentration 1000 µg/ mL HTZ and 1600 µg/ mL OLM. Aliquot of the standard solution was appropriately diluted with the mobile phase containing Acetonitrile, methanol and buffer in the ratio 20:20:60 v/v to get the concentration of 40µg/mL for HTZ and 64µg/mL for OLM respectively.

Procedure

The optimized chromatographic condition mentioned below was kept constant throughout the experimentation and mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.

Column - Phenomenex ODS 5 µ C18 column (250 X 4.6mm)
Detection Wavelength - 230 nm
Flow rate - 1.0 mL/min
Temperature: Ambient - (28-30^{0} C)
pH - 2.2

A 20 µL solution of above mix standard was injected through manual injector and chromatogram was recorded using mobile phase containing Acetonitrile, Methanol and Buffer (20: 20: 60). Hydrochlorothiazide and

ISSN: 0975-8585

Olmesartan medoximil were resolved properly with sharp peak and showing reasonable retention time in the above selected mobile phase. A chromatogram for both drugs so recorded in shown in fig 1.

Study of system suitability parameters

After equilibration of column with mobile phase, seven replicate injections of 20 µL solution of mix standard solution was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in Table 1.

Study of Linearity Range

Aliquots of mixed standard stock solution were diluted in range 1.0 mL to 5.0 mL in 50 mL volumetric flask with mobile phase, volume was made up to mark with mobile phase to obtain concentration 20µg/mL to 100µg/mL for HTZ and 32µg/mL to 160µg/mL for OLM respectively. The graphs of concentration of drug vs. area under curve were plotted for both the drugs. The correlation coefficient was found to be 0.9989 for HTZ and 0.9992 for OLM.

Assay in Marketed Formulation

An accurately weighed quantity of tablet powder equivalent to 10.0 mg of HTZ (~ 16.0 mg of OLM) was transferred to 25.0 mL volumetric flask, sonicated for 30 minutes with sufficient quantity of methanol and volume was made up to mark with methanol. The contents of the flask were filtered through Whatmann filter paper (no.41). A 5.0 mL portion of the filtrate was further diluted to 50.0 mL with a mobile phase. The sample solution was injected and the chromatogram was recorded. The content of HTZ and OLM were calculated by comparison of the standard area and sample area and results are shown in Table 2.

Validation

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 proposed method and the mean % recovery were found to be 100.62 and 99.42 for HTZ and OLM respectively.

Stability studies: The forced degradation studies were carried out at 50^0 C using 1ml of 1N NaOH, 1N HCL, 6% H_2O_2 and the chromatograms recorded are shown in fig 2,3,4 respectively. Volumes were made up to the mark with methanol, further aliquots were diluted with mobile phase and sample solutions were injected separately and chromatograms under stress conditions were recorded. The results showed slight difference in the percent label claim as compared with normal condition. In all the stress condition Olmesartan was found to be more sensitive to hydrolysis and oxidation. The results are shown in Table 3.

Precision and Intermediate precision (Intra day and Inter day) shows the % Label claim values within limits (% R.S.D. not more than 2). The method was found to be précised.
The ruggedness studies were carried out using different analyst variation. The results of intermediate precision and ruggedness parameter are shown in Table 4.

Linearity and Range: Accurately weighed quantities of tablet content equivalent to about 80, 90, 100, 110 and 120% of label claim of HTZ were taken and dilutions were made as described under marketed formulation. The chromatograms of the resulting solutions were recorded. The plot of AUC Vs Percent label claim was found to be linear with correlation coefficient of 0.9976 for HTZ and 0.9984 for OLM.

ISSN: 0975-8585

Table 1 System Suitability Parameter

Parameter	Suitable Values	
	HTZ	OLM
Mobile phase	[Acetonitrile, Methanol and Phosphate Buffer (20:20:60)]	
Retention time:	6.503	8.613
Asymmetry:	1.115	1.581
Resolution:	----	3.468
Efficiency (tl/m):	33374	65580

Table 2 Results of Marketed formulations and Recovery study

Drug	Mean of % label claim* ± S.D.	Mean % Recovery** ± CV
HTZ	101.31 ±1.80	100.62 ± 1.01
OLM	98.64 ±1.50	99.42 ± 0.81

*Mean of five observations, ** mean of four observations

Table 3 Forced degradation study

	% label claim			
Condition	No treatment	1N NaOH	1N HCl	6% H_2O_2
HTZ	97.92	84.23	83.20	75.91
OLM	98.62	100.32	90.15	94.87

Table 4 Intermediate precision and Ruggedness study

Parameters	Mean % label claim ±S.D.	
	HTZ	OLM
Different Analyst (n=3)	100.38 ±0.54	100.65±0.45
Intraday Variation (n=3)	99.95±0.32	100.66 ±0.97
Inter day Variation (n=3)	99.25 ±0.85	98.87±1.59

ISSN: 0975-8585

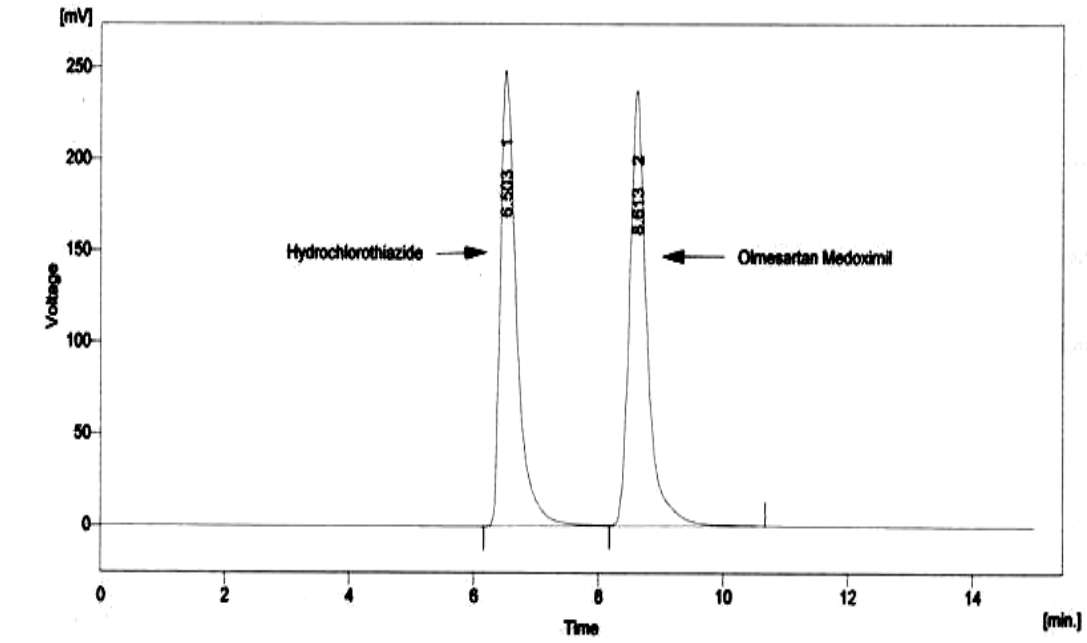

Fig 1 Chromatogram for Hydrochlorothiazide and Olmesartan medoximil

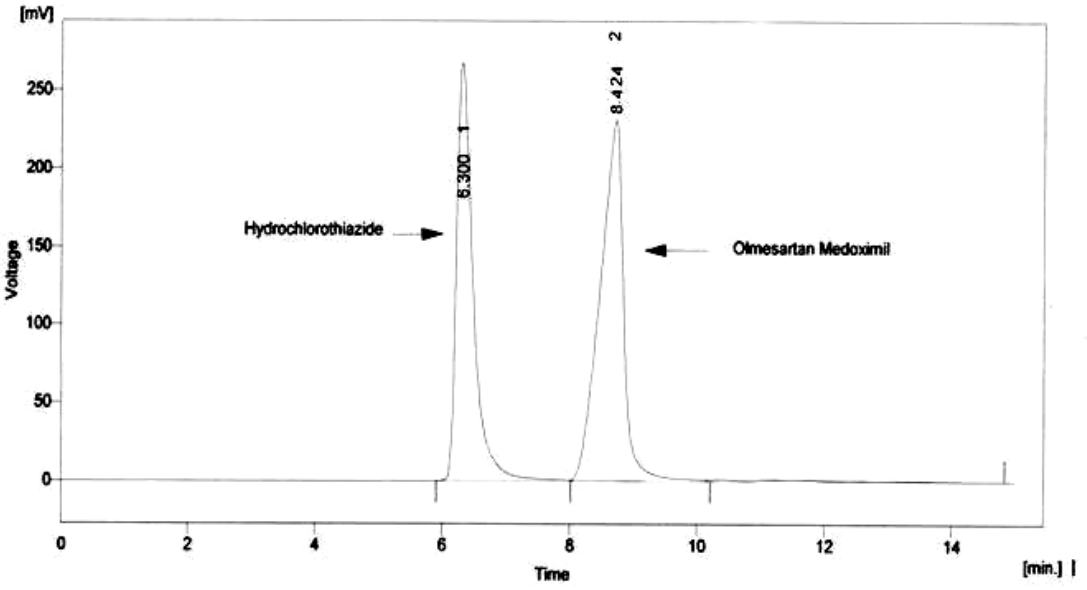

Fig 2 Chromatogram of HTZ and OLM (1N NaOH)

ISSN: 0975-8585

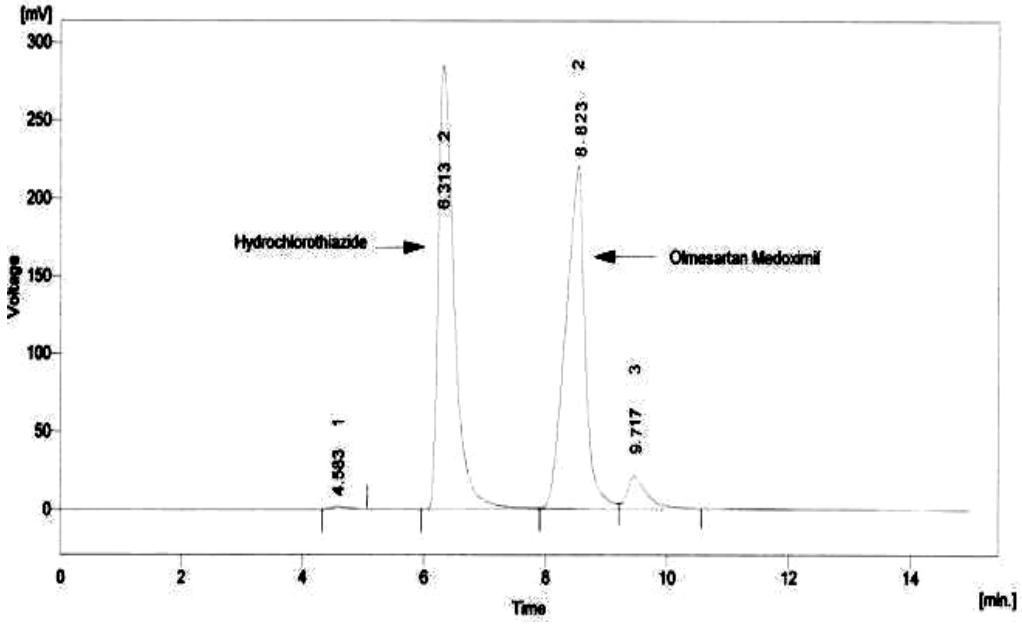

Fig 3 Chromatogram of HTZ and OLM (1N HCl)

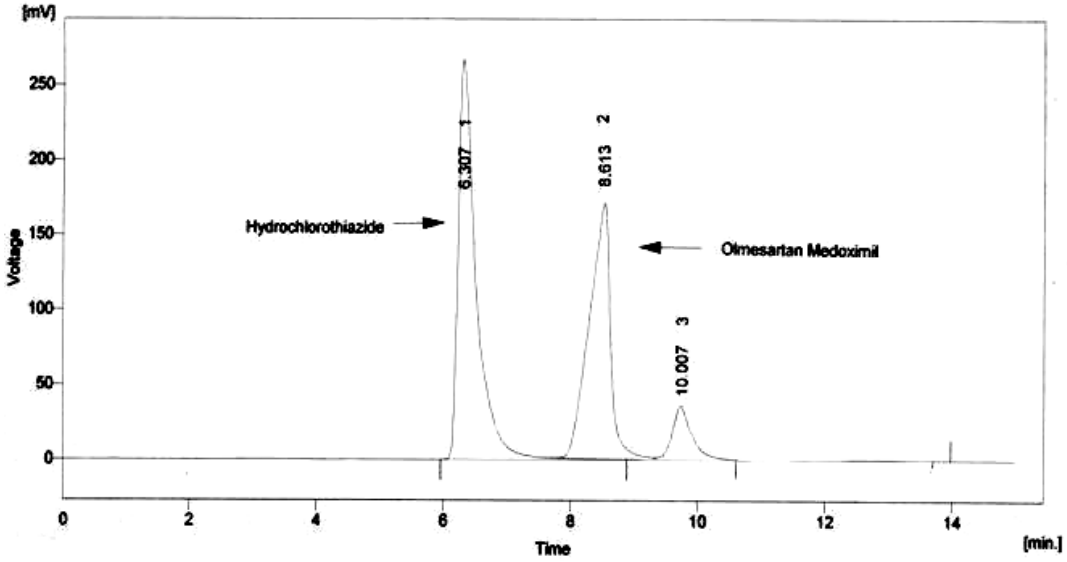

Fig 4 Chromatogram of HTZ and OLM (6% H_2O_2)

ISSN: 0975-8585

RESULTS AND DISCUSSION

The optimised chromatographic conditions gave well resolved and sharp peaks of HTZ and OLM with retention times 6.503 and 8.613 respectively. It was observed that the proposed method can be easily applied to marketed formulation and the statistical parameter viz. S.D., CV is in the acceptable range for quantitative determination of OLM and HTZ. The method validation parameter like accuracy, precision, Linearity and range and specificity were found to be satisfactory.

CONCLUSION

The results obtained by the proposed method for determination of HTZ and OLM are reliable, accurate and precise. The values of standard deviation were found satisfactory and the recovery studies were close to 100%. The method does not require prior separation of one drug from other. Hence it can be employed for routine quality control analysis of HTZ and OLM in combined dosage form.

ACKNOWLEDGEMENTS

The authors are thankful to Alembic Pharmaceutical Ltd. for providing standard drug samples and also to S.K.B.College of Pharmacy, Kamptee for providing the facilities to carry out the work.

REFERENCES

[1] "Indian Pharmacopoeia"; Vol. II Govt. of India, Ministry of Health and Family Welfare, Published by controller of publication New Delhi, 1996 : 371

[2] Adele M. and Neil JO.; ed., "The Merck index" 13th Edn, published by Merck research lab, division of Merck & Co, White house station, NJ, USA, 2001: 6909

[3] Sachin A, Jain DK and Trivedi P. Indian Drugs 1997; 34:168-171.

[4] Liu D, Hu P, Matsushima N, Li X, Li L, and Jiang JJ. Chromatogr. B. Analyt Technol Biomed Life Sci 2007;1-2: 190-197.

[5] Celebier M and Altinos S. Pharmazie 2007;62: 419-22.

[6] Mustafa C and Sacide AJ, Chromatgraphia 2007; 66: 127.

[7] Wankhede SB, Gupta KR, Tajne MR .and Wadodkar SG. Ind J Pharm Sci 2007;69: 298-300.

[8] Vetuschi C, Giannandrea A, Carlucci G and Mazzeo P. Farmaco 2005; 60: 665-670.

[9] Kadam BR and Bari SB. Acta Chromatographia 2007;18: 67.

[10] Ivanovic D, Medenica M, Malenovic A and Jancic B, Accredit. Qual Assur 2004; 9: 76-81

[11] Chintan VP, Amit PK, Anandi DC and Kalpesh TP. Eur J Anal Chem 2007; 2: 57

[12] Shah NJ, Suhagia BN, Shah RR and Patel NM. Ind J Pharm Sci 2007;69: 834-836