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SIMULTANEOUS DETERMINATION OF OLMESARTAN AND HYDROCHLOROTHIAZIDE IN COMBINED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of Olmesartan and Hydrochlorothiazide in bulk and pharmaceutical dosage forms. Chromatography was carried out by using Chromosil C-18,column having 250 x 4.6mm internal diameter with a mixture of methanol, acetonitrile and TEA in the ratio of 46:50:04 (v/v/v) as mobile phase. Determination of the different analytical parameters such as linearity, precision, accuracy, and specificity, limit of detection (LOD) and limit of quantification (LOQ) was done. The calibration curve was found to be linear for each analyte in the desired concentration range. The % recovery was found to be 99.59 and 99.61 for Olmesartan and Hydrochlorothiazide respectively. The proposed method is highly sensitive, precise and accurate, which was evident from the LOD value of 0.05 and 0.02 ppm for Olmesartan and Hydrochlorothiazide respectively and hence the present method can be applied successfully for the quantification of active pharmaceutical ingredient (API) content in the combined formulations of Olmesartan and Hydrochlorothiazide

KEY WORDS

Olmesartan ,hydrochlorothiazide ,RP-HPLC Method

INTRODUCTION

Olmesartan is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents.[4] The U.S. Food and Drug Administration (FDA) has determined that the benefits of Benicar continue to outweigh its potential risks when used for the treatment of patients with high blood pressure according to the drug label.[5] Contraindications for treatment with olmesartan include biliary obstruction (BNF). Angiotensin-II receptor antagonists should be used with caution in renal artery stenosis. Monitoring of plasma-potassium concentration is advised, particularly in the elderly and in patients with renal impairment; lower initial doses may be appropriate in these patients. Angiotensin-II receptor antagonists should be used with caution in aortic or mitral valve stenosis and in hypertrophic cardiomyopathy. Those with primary aldosteronism, and Afro-Caribbean patients (particularly those with left ventricular hypertrophy), may not benefit from an angiotensin-II receptor antagonist. The incidence of adverse effects with BENICAR (the trade name for olmesartan medoxomil) is reported as similar to placebo; the only adverse effect that occurred in >1% of patients treated with BENICAR and more frequently than placebo was dizziness (3% vs 1%). The full prescribing information for Benicar notes that as with all drugs that act directly on the renin-angiotensin system, olmesartan is contraindicated in pregnancy and can cause injury and even death to the developing fetus. In studies of angiotensin II receptor antagonists such as olmesartan, patients with unilateral or bilateral renal artery stenosis, increases in serum creatinine or blood urea nitrogen (BUN) have been reported. There has been no long-term use of olmesartan medoxomil in patients with unilateral or bilateral renal artery stenosis, but similar results may be expected.[6]

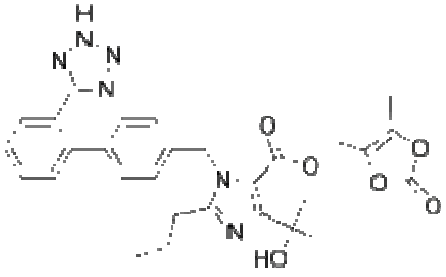


Figure.1
structure of Olmesartan

Hydrochlorothiazide is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones.[6] It is also sometimes used for hypercalciuria, Dent's disease and Ménière's disease. For diabetes insipidus, the effect of thiazide diuretics is presumably mediated by a hypovolemia-induced increase in proximal sodium and water reabsorption, thereby diminishing water delivery to the ADH-sensitive sites in the collecting tubules and reducing the

urine output.Thiazides are also used in the treatment of osteoporosis. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney, and by directly stimulating osteoblast differentiation and bone mineral formation.[7]is a first-line diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance.[5] Hydrochlorothiazide is a calcium-sparing diuretic, meaning it can help the body get rid of excess water while still keeping calcium.

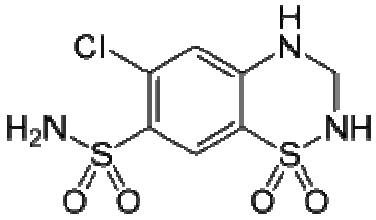


Figure 2
Structure of Hydrochlorothiazide

EXPERIMENTAL

Chemicals and Reagents

Olmesartan and Hydrochlorothiazide as pure standard reference drugs were purchased from Reddy's Laboratory, Hyderabad and pharmaceutical formulation from local market were used for this present study. Water, Acetonitrile, methanol and orthophosphoric acid,Tri Ethyl Amine (all HPLC grade) were purchased from Merck Specialties Private Limited, Mumbai, India.

Instrumentation

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Hydrochlorothiazide and Olmesartan, an isocratic PEAK HPLC instrument with Hypersil C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software. UV-2306 Spectrophotometer was used for wavelength checking. Denver analytical Balance was used to weigh the drug.

Experimental Condition

Flow rate of the mobile phase was changed from 0.5 – 1.5 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 ml/min flow rate was ideal for the successful elution of the analyte. The HPLC system was hence operated using an isocratic mode at a flow rate of 1.0 ml/min at 25 ± 2^{oc}. For analysis the most suitable mobile phase was found to be Methanol, Acetonitrile and TEA in the ratio of 46:50:04. Detection was carried out at wavelength of 259 nm.

Preparation of Mobile Phase

For the preparation of mobile phase suitable for the present determination methanol, acetonitrile and TEA acid of HPLC grade were mixed, filtered and degassed in such a way that the final volume consisted of these in the ratio 46:50:04 respectively, whose pH was adjusted to 6.8.

Preparation of mixed standard solution

Olmesartan and Hydrochlorothiazide (1mg/ml) standard stock solutions were prepared using methanol as a solvent. Aliquots of mixed standard solutions of Olmesartan and

Hydrochlorothiazide were diluted in mobile phase to get a final concentration of 40-100ppm.

Preparation of sample solution of pharmaceutical formulation

Pharmaceutical form containing 8 mg of Olmesartan and 12.5 mg of Hydrochlorothiazide was weighed and dissolved in 25 ml of methanol and sonicated for 15 min. Using methanol the volume was made up to 50 ml and filtered through 0.45µ membrane filter. The final mixed sample solution corresponding to 40 ppm of Olmesartan and 60.25 ppm of Hydrochlorothiazide was prepared.

Recording of chromatograms

After stabilization of the base line with the optimized chromatographic conditions standard solutions containing 40-100 ppm of Olmesartan and Hydrochlorothiazide were injected and the corresponding chromatograms were recorded. Retention time of Olmesartan and Hydrochlorothiazide were found to be 1.5 and 3.5 mins respectively. Likewise for sample solution chromatograms were recorded. Calibration curves were plotted using peak area retentions of standard drug peaks against concentration of corresponding standard solutions.

RESULTS AND DISCUSSION

Method validation

The method was validated by determining linearity, precision, accuracy, specificity, ruggedness and robustness by analyzing 40-100 ppm of both Olmesartan and Hydrochlorothiazide.

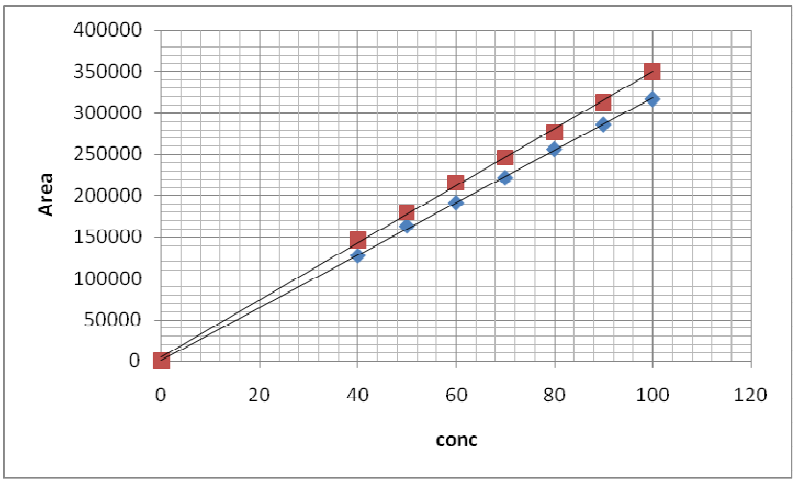
Table 1

Optimized chromatographic conditions for estimation of Olmesartan and Hydrochlorothiazide

Mobile phase	Methanol : Acetonitrile:TEA 46:50:04 (v/v/v)
Pump mode	Isocratic
A.P.I Conc	Olmesartan - 7 PPM Hydrochlorothiozide - 7 PPM
pH	6.8
Diluents	Mobile phase
Column	C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	259 nm
Injection Volume	20 µL
Flow rate	1.0 mL/min
Run time	10 minutes
Retention Time	Olmesartan – 1.5 Hydrochlorothiozide – 3.5

Linearity

The linearity of the response for Olmesartan and Hydrochlorothiazide assay method was determined by preparing and injecting standard solutions of Olmesartan and Hydrochlorothiazide. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied with correlation coefficient (r^2) value, slope and intercept as shown in table 3.



Graph 1
Calibration Plot for Olmesartan and Hydrochlorothiazide

Table.2

S.NO	CONCENTRATIONS	Olmesartan peak area	Hydrochlorothiazide peak area
1	**40**	127605	146522
2	**50**	163545	180158
3	**60**	191473	215992
4	**70**	221862	246402
5	**80**	256579	276930
6	**90**	286424	313269
7	**100**	317192	350243

Table 3
Regression analysis of the calibration curve

Parameters	Olmesartan	Hydrochlorothiazide
Calibration range (ppm)	40-100	40-100 ppm
Slope	3172.797	3459.117
Intercept	1251.173	4318.556
Correlation coefficient (r^2)	0.9998	0.9995

Precision

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Olmesartan and Hydrochlorothiazide combined test solution in the same equipment at a concentration value of 70 ppm on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak

areas of the drugs were determined and precision as % RSD was reported.

Table.4
Intraday precision

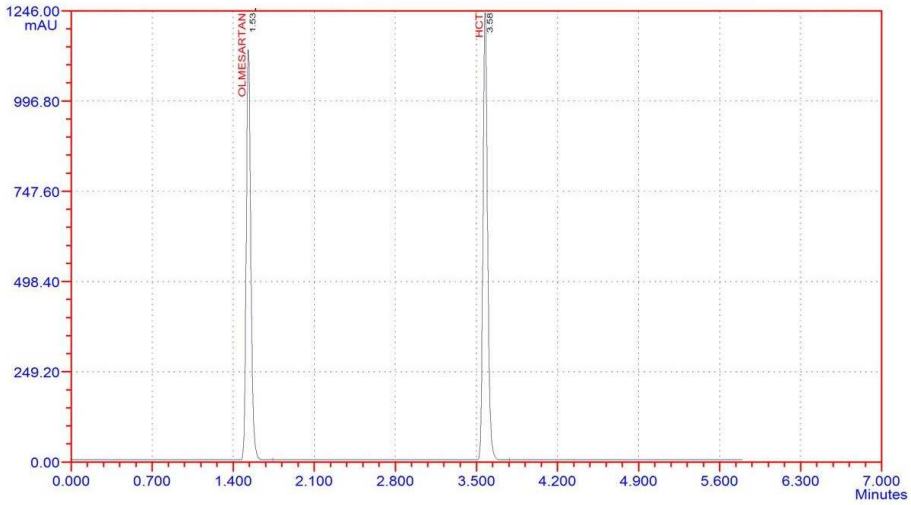
S.NO	CONCENTRATION	**Olmesartan peak area**	**Hydrochlorothiazide peak area**
1	70 PPM	221884	246202
2	70 PPM	221015	246589
3	70 PPM	221328	246397
4	70 PPM	221492	246571
5	70 PPM	221030	246056
6	70 PPM	221563	246959
		%R.S.D = 0.15	%R.S.D = 0.154

Table.5
Interday precision

S.NO	CONCENTRATION	**Olmesartan peak area**	**Hydrochlorothiazide peak area**
1	70 PPM	221386	246176
2	70 PPM	221934	246372
3	70 PPM	221058	246087
4	70 PPM	221943	246905
5	70 PPM	221683	246712
6	70 PPM	221708	246534
		%RSD = 0.154	%RSD = 0.127

Table 6.
System suitability and validation parameters

Parameters	**Olmesartan**	**Hydrochlorothiazide**
Theoretical plates (N)	6017	32282
Retention time (min)	1.5	3.5
Tailing factor	1.41	1.39
LOD (ppm)	0.05	0.02
LOQ (ppm)	0.15	0.08
R.S.D. (%)	0.152	0.14



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	OLMESARTAN	1.532	113781	317192.9	47.524	1.41	6017
2	HCT	3.577	124582	350243.1	52.476	1.39	32262
	Sum:		238363	667436.0	100.0000		

Figure.3

Typical chromatogram of standard Olmesartan and Hydrochlorothiazide

Recovery

The recovery of the standard solutions was done by adding them to pre-analyzed sample solution at different levels i.e. 50%, 100%, and 150% separately to study the accuracy of the above method. The corresponding results were recorded.

Table.7

Recovery of Olmesaartan, Hydrochlorothiazide

S.NO	CONCENTRATION In ppm	Olmesartan AMOUNT RECOVERED	Hydrochlorothiazide AMOUNT RECOVERED	%of Olmesartan RECOVERED	% of Hydrochlorothiazide RECOVERED
1	40	39.9	39.7	99.75	99.25
2	60	59.8	59.3	98.83	99.83
3	80	79.96	80.01	99.95	100.012
				Avg Recovery =99.51	Avg Recovery =99.69

Specificity

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Olmesartan and Hydrochlorothiazide. The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Olmesartan and Hydrochlorothiazide.

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

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guidelines.

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test described above.

. **Table.8**

Robustness study

S.NO	PARAMETER	CONDITION	Olmesartan peak area	Hydrochlorothiazide peak area
1	Standard	Standard conditions	221386	246176
2	Mobile phase	Methanol 40%,ACN 50%,TEA10	221453	246871
3	Mobile phase pH	6.6	221867	246374
4	Wavelength	261 nm	221069	246985

Analysis of marketed formulations

The validated HPLC method was adopted for the quantification of Olmesartan and Hydrochlorothiazide in their combined pharmaceutical dosage form and the typical chromatograms of the formulation are shown in fig. The results of analysis are given in Table 8. The contents of the pharmaceutical dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of Olmesartan and Hydrochlorothiazide in pharmaceutical dosage form.

Table.9

Formulation

S.NO	Drug	Dosage	Sample Conc.	Amount of drug estimated	% of drug estimated
1	Olmesartan	8 mg/ml	40ppm	39.92ppm	99.8
2	Hydrochlorothiazide	12.5mg/ml	60.25 ppm	60.21 ppm	99.93

CONCLUSION

The proposed study describes a new RP-HPLC method using simple mobile phase for the estimation of Olmesartan and Hydrochlorothiazide in combined pharmaceutical dosage formulations. The method was validated and found to be simple, sensitive, accurate and precise. It was also proved to be convenient and effective for the determination of Olmesartan and Hydrochlorothiazide in the pharmaceutical dosage form. The percentage of recovery shows that the method is free from interference of the excipients used in formulation. Moreover, the

lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method.

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