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Development and validation of RP-HPLC and UV-Spectrophotometric method for olmesartan medoxomil and hydrochlorthiazide in combined dosage form

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ABSTRACT

A reverse phase-high performance Liquid chromatography method is a simple, accurate, precise and reproducible one. UV-Spectrophotometric simultaneous equation method is adopted by official compendia for the stable substance that have reasonably broad absorption bands and which are practically unaffected by the variations of Instrumental parameters. The use of standard $A(1\%1\text{cm})$ value avoids the need to prepare a standard solution of the reference substance in order to determine absorptivity. A reverse phase high performance liquid chromatography method has been developed for the simultaneous estimation of Olmesartan medoxomil and Hydrochlorthiazide acid in tablet dosage form using C18 column (LC 20 AT Isocratic) in Isocratic mode. The mobile phase consisted of Acetonitrile, methanol and 20 μl phosphate buffer adjusted to PH 3.5 in ratio of 60:20:20 v/v with ultraviolet visible detection at 230 nm. The method was linear over the concentration range for Olmesartan medoxomil 5 - 70 $\mu\text{g/ml}$ and for Hydrochlorthiazide 5 - 50 $\mu\text{g/ml}$. The mean recovery was found to be in the range of 98% to 102%. The Validation method was carried out using International Conference on Harmonisation Guidelines. The described RP-HPLC method was successfully employed for the analysis of Pharmaceutical formulations containing combined dosage form.

Key words:- Simultaneous estimation, RP-HPLC, Olmesartan medoxomil, Hydrochlorthiazide, ICH-guidelines.

INTRODUCTION

Olmesartan medoxomil is a 4-(1-hydroxy-1-methyl ethyl)-2-propyl-1-[(2-(1H-tetazol-5-yl){1,1'-biphenyl}-4-yl)methyl]-1-H-imidazole-5-carboxylic acid(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester..(STRUCTURE-1a) with the Antihypertensive activity by preventing angiotension-

II to binding AT-1 receptors. Hydrochlorothiazide is chemically 3,4-dihydro-6-chloro-7-sulfamoyl-1,2,4-benzothiadiazine-1,1-dioxide;6-chloro-3,4-dihydro-7-sulfamoyl-1-2H-1,2,4-benzathiadiazine-1,1-dioxide;3,4-dihydrochlorothiazide(STRUCTURE-1b) is a diuretic effect. A combination of these drugs containing Olmesartan medoxomil and Hydrochlorothiazide is commercially available and more effective and had a high safety profile in the treatment of Hypertension and edema.

Literature review indicates that various analytical methods like High Performance Thin Layer Chromatography, HPLC and Spectrophotometric methods have been reported for the determination of Olmesartan medoxomil and Hydrochlorothiazide from their formulations individually and in combination with other drugs. The literature review indicates that no method is yet reported for the simultaneous estimation of both drugs in combination. This prompted us to develop a simple, accurate, precise and sensitive simultaneous estimation of Olmesartan medoxomil and Hydrochlorothiazide by RP-HPLC and spectrophotometric methods. The method was validated as per ICH-guidelines.

MATERIALS AND METHODS

Drugs and chemicals:-

The Pharmaceutical grade pure samples of Olmesartan medoxomil(99.26%) and Hydrochlorothiazide(99.65%) was supplied by DR.CEEL ANALYTICAL LAB, CHENNAI, INDIA. aceto nitrile, methanol HPLC-grade solvents and all analytical grade solvents obtained from E-merk limited, Mumbai, India. Sodium dihydrogen orthophosphate analytical grade reagent was procured from Qualigens fine chemicals, Mumbai, India. The HPLC-grade water was obtained from a milli-Q -water purification system.

HPLC- apparatus and condition:-

The separation was performed by using C18 (250×4.6mm,5µm) column on a shimadzu LC 20 AT Isocratic solvent delivery system. Shimadzu SPD-10A dual wavelength absorbance detector and Rheodyne injector with 20mM phosphate buffer(adjusted to PH-4). Acetonitrile : methanol in ratio of (60:20:20v/v) were used. The mobile phase was freshly prepared, filtered and sonicated before use and delivered at a flow rate of 1.0 ml/min and the detector wave length was set at 230 nm. The injection volume was 20µl(fixed loop).

Stock solution and standard:-

Standard stock solution were prepared for 1000µg/ml by using Olmesartan medoxomil and Hydrochlorothiazide separately by using mobile phase. From the standard stock solution different concentrations of working standards were prepared from the range of 5 to 70µg/ml for Olmesartan medoxomil and 5 - 50µg/ml for Hydrochlorothiazide.

Calibration curve:-

The calibration curve were constructed for the determination of the linearity and the curves were plotted with the concentration range verses area must obey the Beer's law. The linearity was evaluated by the analysis of serially diluted sample in the range of 5 - 70µg/ml for Olmesartan medoxomil and 5 - 50µg/ml for Hydrochlorothiazide. An aliquot was injected by using mixture of 20mM Phosphate buffer:Acetonitrile:Methanol(60:20:20v/v). The 20µl mixture was injected

for the estimation under the optimized chromatographic conditions. The typical chromatogram was recorded for standard as shown in figure-1. The retention time of standard Olmesartan medoxomil and Hydrochlorothiazide were found to be 4.3 min and 3.1 min respectively with a good resolution.

Analysis of formulation:-

Twenty tablets were weighed and finely powdered. A quantity equivalent to 20 mg Olmesartan medoxomil and 12.5 mg Hydrochlorothiazide were transferred in to 100ml volumetric flask and dissolved on about 50ml mobile phase. The solution was ultrasonicated for 10 minutes and filtered through 0.45 μ nylon membrane and degassed and the volume was made upto mark with same system. Above solution was taken to prepare a dilution of 200 μ g/ml Olmesartan medoxomil and 125 μ g/ml Hydrochlorothiazide. The amount of drug was determined and three replicate injections were done.

RESULTS AND DISCUSSIONS

Method development:-

Several tests were performed in order to get satisfactory separation and the resolution of Olmesartan medoxomil and Hydrochlorothiazide in different mobile phases with various ratios of organic phase and buffers by using C18 column. The ideal buffer was 20mM phosphate buffer (PH-4): Acetonitrile :Methanol in ratio(60:20:20v/v) by Isocratic elution to obtain satisfactory and good resolution. The changes in PH of mobile phase by ± 0.2 does not shows any significant change in retention time of each analyte. The retention of Olmesartan medoxomil and Hydrochlorothiazide on analytical column was evaluated at the flow rate of 1.0 ml/min and the Injection volume was 20 μ l. The retention time of standard and sample for Olmesartan medoxomil and Hydrochlorothiazide were satisfactory with good resolution.

Linearity:-

The linearity for HPLC method was determined at five concentration levels. The linearity of Olmesartan medoxomil and Hydrochlorothiazide were determined by calibration curves and the linearity based on the area observed in the range of 160-240 μ g/ml for Olmesartan medoxomil and 100-150 μ g/ml for Hydrochlorothiazide. The % relative standard deviation (%RSD) of peak area and the retention time was within the limit of $\pm 0.2\%$. This indicates that, the method was system suitable. The reports are tabulated in Table-1.

Table -1. System Suitability Parameters

PARAMETERS	Olmesartan medoxomil	Hydrochlorothiazide
Calibration range(μ g/ml)	5 - 70	5 - 50
Correlation Co-efficient(r^2)	0.9994	0.9999
Retention time(min)	4.3 \pm 0.2	3.1 \pm 0.2
Resolution	6.4	6.4
Repeatability(%RSD)(n=5)	0.337%	0.332%
Theoretical plates	8797	4280
Tailing factor	1.444	1.783
Limit of quantification(μ g/ml)	9.6 μ g/ml	2.8 μ g/ml

The regression co-efficient value(r^2) for Olmesartan medoxomil and Hydrochlorothiazide is 0.9999 and 0.9998 respectively.

Precision:-

Precision was measured for both inter and intra-day and checked with repeatability and the %RSD for the repeatability was found to be 0.496% and 0.225% respectively for Olmesartan medoxomil and Hydrochlorothiazide. The % RSD was found within the limit and was tabulated in Table-1. The limit of quantification was determined by injecting minimum concentration of the drugs. The limit of quantification was found to be 9.6µg/ml for Olmesartan medoxomil and 2.8µg/ml for Hydrochlorothiazide.

Recovery Studies:-

The assay procedure was repeated for standard and sample in five times and mean peak area ratio and concentration of drugs were calculated. The percentage of individual drugs found in formulation, mean and % RSD in formulation were calculated and shown in Table-2. Recovery studies carried out for both drugs. It is usually done by adding 80%, 100%, and 120% of the pure drug with the formulation taken for analysis. The average % recovery for Olmesartan medoxomil and Hydrochlorothiazide was found to be 99.99% and 99.00% respectively. The results were represented in table-3.

Specificity and selectivity:-

Specificity was tested against standard compounds and potential interferences. To determine specificity with respect to sample compounds the response of standard and sample solution were compared. No interferences were detected at the retention time of either Olmesartan medoxomil or Hydrochlorothiazide in sample solution. The limit of detection (LOD) was determined at lowest concentration giving response and limit of quantification was determined at the lowest concentration. The limit of detection (LOD) for Olmesartan medoxomil and Hydrochlorothiazide was found to be 3.1µg/ml and 0.9µg/ml respectively. The limit of quantification (LOQ) was 9.6µg/ml for Olmesartan medoxomil 2.8µg/ml for Hydrochlorothiazide and was given in Table-1.

Table-2 Analysis of Marketed Formulations

Formulations	Olmesartan medoxomil			Hydrochlorothiazide.		
	Label Claim (mg/tab)	Amount Found* (mg/tab ±RSD)	% Assay±RSD	Label Claim (mg/tab)	Amount Found* (mg/tab±RSD)	% Assay±RSD
ASMR	20	99.90±0.368	99.9±0.896	12.5	12.35±0.385	98.87±0.875

*stands for the average reading taken in three reading

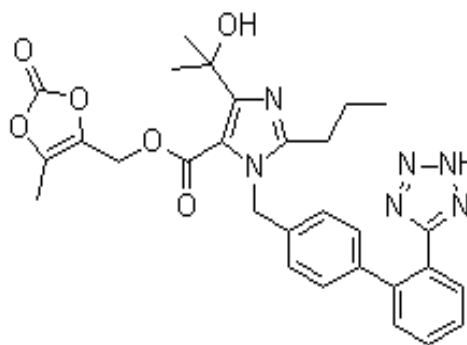
Table-3 Recovery studies of olmesartan medoxomil and hydrochlorothiazide in combined dosage form

Formulation	Olmesartan medoxomil.			Hydrochlorothiazide.		
	% added	%recovered* ±RSD	%recovery ±RSD	%added	%recovered* ±RSD	%recovery ±RSD
Brand.	80	0.1104	99.80±0.014	80	0.1011	98.99 ±0.382
	100	0.1192	99.99±0.013	100	0.1015	98.82±0.010
	120	0.0995	99.99±0.064	120	0.1929	99.00±0.201

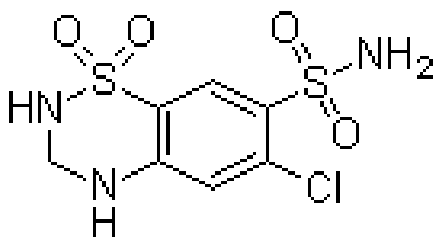
* Recovery experiment data for Mefanamic acid and Drotaverine hydrochloride showing the amount of drug recovered from sample solution at each level(n=3), percentage recovery and the average percentage recovery.

Stability:-

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analysed over a period of 24 hours at room temperature. The results found for both solutions. The retention time and peak area of Olmesartan medoxomil and Hydrochlorothiazide remain almost similar (% RSD less than 2.0) and significant degradation within the indicated period, this indicates that both solutions were sufficient to complete the whole analytical process.

STRUCTURES:

1(a): Structure of Olmesartan medoxamol.



1(b) : Structure of Hydrochlor thiazide

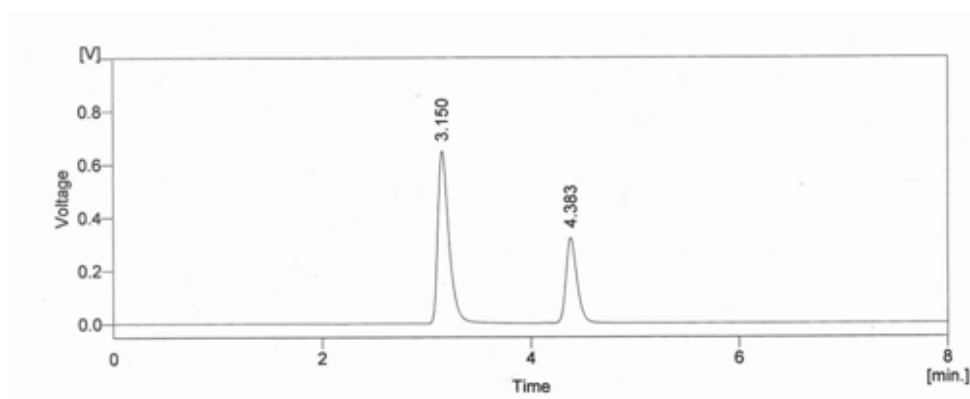
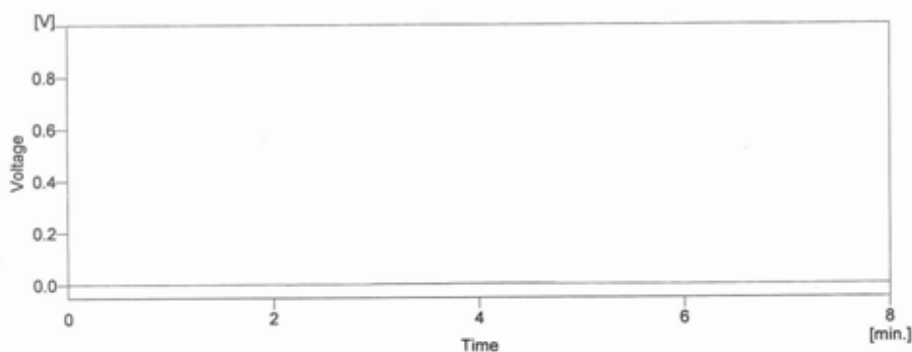
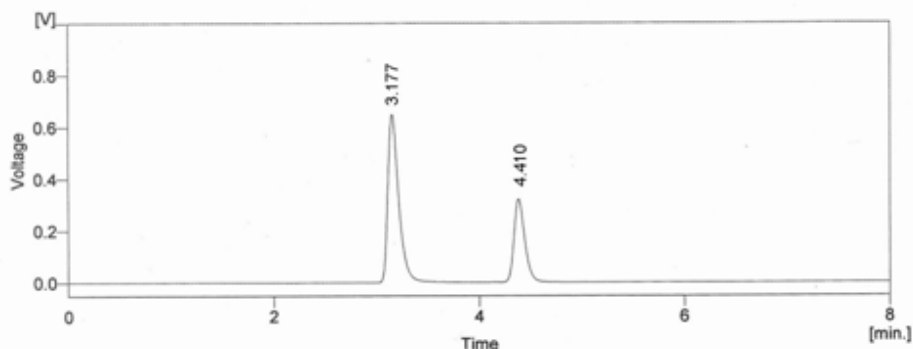


Fig 1 : A Typical Chromatogram For Olmesartan medoxomil and Hydrochlorthiazide

**Fig 2(a) : Blank****Fig 2(b): Linearity at 100% level.****Ruggedness and robustness:-**

Ruggedness test was determined by different analyst in different days using similar operational environmental conditions. Robustness of the method was determined by changing the wavelength and flow rate. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

CONCLUSION

Olmesartan medoxomil and Hydrochlorothiazide combined tablet dosage form was analysed by UV-Spectrophotometric simultaneous equation and reverse phase high performance liquid chromatography. On comparing these methods, RP-HPLC method was found to be more precise, accurate, rugged, robust, simple and rapid than UV-Spectrophotometric method and suitable for the quality control of the raw materials, formulations, dissolution studies and also for bioequivalence studies of the same formulations.

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