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Research Article

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A new and rapid analytical method development & validation for simultaneous estimation of hydrochlorothiazide, amlodipine & olmesartan in tablet dosage form by using RP-HPLC

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ABSTRACT

In the present work a new and accurate RP-HPLC method was developed and validated for simultaneous estimation of Hydrochlorothiazide, Amlodipine & Olmesartan in bulk drug and in combined dosage forms. The HPLC separation was achieved on a Symmetry C_{18} (4.6 X 150mm, 5µm, Make: XTerra) or equivalent in an Isocratic Mode. The mobile phase was composed of TEA Buffer (40%) whose pH was adjusted to 3.5 by using Ortho Phosphoric Acid & Acetonitrile (60%) [HPLC Grade]. The flow rate was monitored at 0.8ml per min. The wavelength was selected for the detection was 230 nm. The run time was 9min. The retention time found for the drugs Hydrochlorothiazide, Amlodipine & Olmesartan were 3.034 min., 4.062 min. & 5.165 min. respectively. The % recovery was found to be 99.3- 101.7 for Hydrochlorothiazide. The % recovery was found to be 98.3 - 99.3for Amlodipine. The % recovery was found to be 98.3 - 100.7 for Olmesartan. The linearity was established in the range of 25 to 62.5ppm for Hydrochlorothiazide & 10 to25ppm for Amlodipine & 10 to100 ppm for Olmesartan. The LOD for Hydrochlorothiazide, Amlodipine & Olmesartan were found to be 0.009µg/ml, 0.06µg/ml & 0.06µg/ml respectively. The LOQ for Hydrochlorothiazide, Amlodipine & Olmesartan were found to be 0.03µg/ml, 0.2µg/ml & 0.2µg/ml respectively. The proposed method was adequate sensitive, reproducible and specific for the determination of Hydrochlorothiazide, Amlodipine & Olmesartan in bulk as well as in tablet dosage form. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Hydrochlorothiazide, Amlodipine & Olmesartan in bulk drug and in combined dosage forms.

Keywords: Hydrochlorothiazide, Amlodipine, Olmesartan, ICH Guideline, RP-HPLC, LOD, LOQ.

INTRODUCTION

Olmesartan Medoxomile (OLME), chemically (5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl]phenyl} methyl)-1H-imidazole-5-carboxylate, is an angiotensin II receptor blocker used as an antihypertensive agent. In the literature, several analytical methods have been reported for the determination of OLME in biological fluids and pharmaceutical formulations, including liquid chromatography coupled with mass spectrometry, HPLC, HPTLC and Spectrophotometric estimation[1–5]. In these

methods, OLME was analyzed either alone or in combination with other drugs. Amlodipine besylate (AMLO), chemically (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate, is a long-acting calcium channel blocker that is used as an antihypertensive agent [6–9]. For AMLO, alone or in combination, several analytical methods such as HPLC and HPTLC have been reported for its estimation in biological fluids and pharmaceutical formulations [10–12]. Hydrochlorothiazide (HCTZ), chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide, is a diuretic. Various analytical methods have been reported for the analysis of HCTZ alone and in combination in tablet dosage form and for forced degradation studies [13-14]. A literature survey revealed that no method has been reported on these drugs in combined pharmaceutical dosage forms. Therefore, in the present study, an attempt was made to develop a simple, precise, accurate and robust HPLC method on the analysis of OLME, AMLO and HCTZ in bulk and pharmaceutical formulation.

EXPERIMENTAL SECTION

Chromatographic Conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Symmetry C_{18} (4.6 X 150mm, 5µm, Make: XTerra), a 2695 binary pump, a 20 µL injection loop, auto sampler and a 2487 dual absorbance DAD or UV detector and running on Waters Empower software.

Chemicals and solvents: The following chemicals were procured for the process: Water [HPLC Grade], Acetonitrile [HPLC Grade], Methanol [HPLC Grade], Hydrochlorothiazide, Amlodipine & Olmesartan [Working standards], Orthophosphoric Acid & TEA all the chemicals were procured from STANDARD SOLUTIONS and the tablets were collected from the Local market.

Preparation of TEA buffer pH 3.5: The buffer solution was prepared by dissolving accurately weighed 0.1ml of TEA and transferred into a clean and dry 1000ml volumetric flask, dissolved and diluted with 1000ml water [HPLC Grade]. The final pH of the buffer was adjusted to 3.5 with Ortho Phosphoric Acid.

Preparation of mobile phase & diluent: The Mobile Phase was prepared by mixing 400 ml (40%) of the above buffer and 600 ml of Acetonitrile [HPLC Grade] (60%) and degassed in an ultrasonic water bath for 10 minutes. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration. The mobile phase was used as diluent.

Preparation of Standard solution: The standard solution was prepared by weighing accurately 12.5mg Hydrochlorothiazide, 5.0mg Amlodipine & 20.0mg Olmesartan and transferred into clean and dry 10ml volumetric flask. Initially about 7ml of diluent was added to the flask respectively and sonicated. The volume was made upto the mark with the same diluent. From the above prepared Stock solution pipette out 0.4ml of Hydrochlorothiazide, Amlodipine & Olmesartan solution and transferred into a clean and dry 10ml volumetric flask, the diluent was added upto the mark to get final concentration.

Preparation of Sample Solution: The sample solution was prepared by weighing equivalently 190mg of Hydrochlorothiazide, Amlodipine & Olmesartan and transferred into a 10ml clean and dry volumetric flask and about 7ml of diluent was added and sonicated to dissolve it completely and the volume made up to the mark with the same solvent. From above prepared stock solution pipette out 0.4ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark to get final concentration. The standard and sample solutions were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

Calibration Plot: About 12.5mg Hydrochlorothiazide, 5.0mg Amlodipine & 20.0mg Olmesartan were weighed accurately and transferred into clean and dry 10ml volumetric flask, and dissolved with 7 mL of a 40:60 v/v mixture of TEA buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a stock solution. From this, a working standard solution dilutions ranging from 25-62.5ppm for Hydrochlorothiazide, 10-25ppm for Amlodipine and 40-100ppm for Olmesartan were prepared from the solution in 10 mL volumetric flasks using the above diluent. 20 µL of each dilution was injected six times into the column at a flow rate of 0.8 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph

constructed by plotting concentration of the drug against peak area (Fig No. 1, 2 & 3) was found to be linear in the concentration range of 25-62.5ppm for Hydrochlorothiazide, 10-25ppm for Amlodipine and 40-100ppm for Olmesartan. The relevant data are furnished in Table No. 1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Hydrochlorothiazide, Amlodipine & Olmesartan in tablet dosage forms.

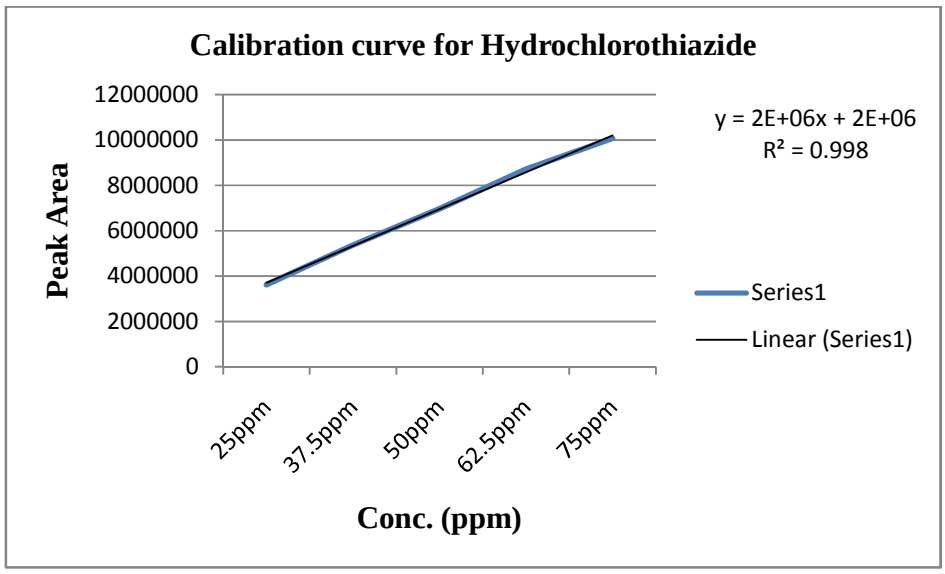

Fig. No.1 Calibration Curve for Hydrochlorothiazide

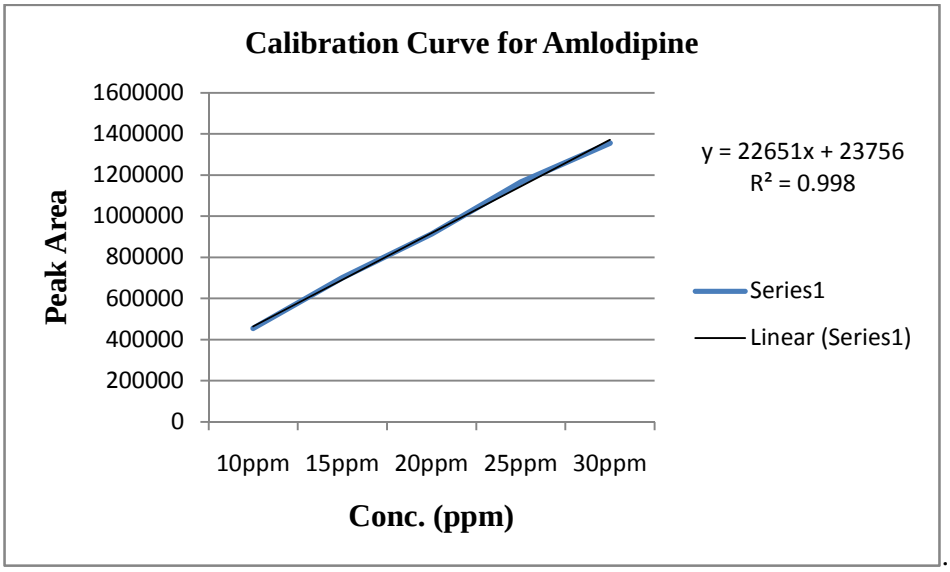

Fig. No. 2 Calibration Curve for Amlodipine

Procedure: A mixture of TEA buffer pH 3.5 and acetonitrile in the ratio of 40:60 v/v was found to be the most suitable mobile phase for ideal separation of Hydrochlorothiazide, Amlodipine & Olmesartan. The solvent mixture was filtered through 0.45µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.8 mL/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. Inject 20µL of the standard, sample solutions into the chromatographic system and measure the area for the Hydrochlorothiazide, Amlodipine & Olmesartan peak. The detection of the drug was monitored at

230nm. The run time was set at 9min. Under these optimized chromatographic conditions the retention time obtained for the drug Hydrochlorothiazide, Amlodipine & Olmesartan 3.034 min., 4.062 min. & 5.165 min. respectively.

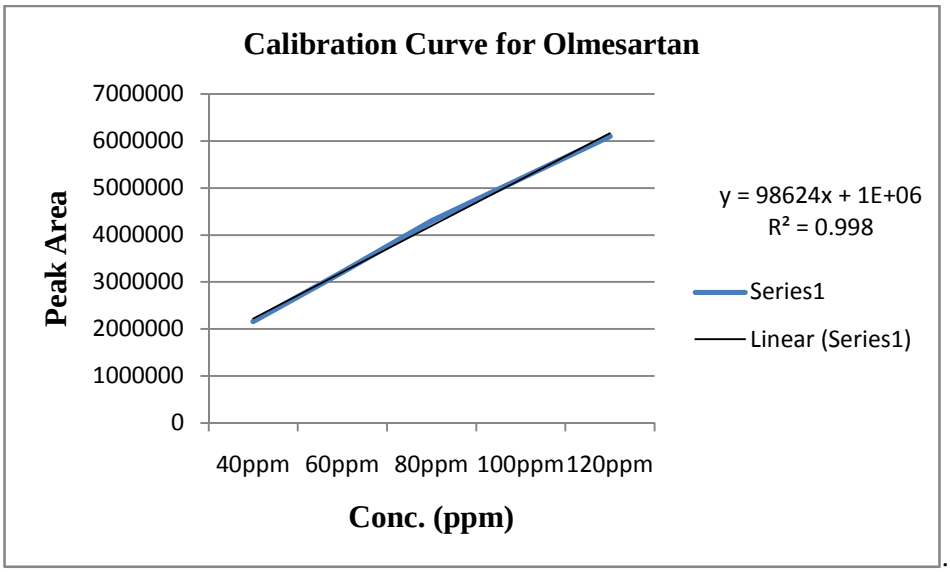

Fig. No.3 Calibration Curve for Olmesartan

Validation of the proposed method: The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method as per the ICH guidelines[15] for the estimation of Hydrochlorothiazide, Amlodipine & Olmesartan. Solution containing desired concentration of Hydrochlorothiazide, Amlodipine & Olmesartan was subjected to the proposed HPLC analysis to check precision of the method and the results are furnished in Table 2. The accuracy of the HPLC method was assessed by analyzing solutions of Hydrochlorothiazide, Amlodipine & Olmesartan at 50%, 100% and 150% concentration levels by the proposed method. The results are furnished in Table 3.

Table No. 1. Calibration data of the method

Linearity Level	Hydrochlorothiazide		Amlodipine		Olmesartan	
	Conc.	Area	Conc.	Area	Conc.	Area
I	25ppm	3610874	10ppm	454409	40ppm	2162681
II	37.5ppm	5354435	15ppm	698979	60ppm	3207767
III	50ppm	6963745	20ppm	913122	80ppm	4296375
IV	62.5ppm	8709208	25ppm	1165154	100ppm	5198765
V	75ppm	10063272	30ppm	1353907	120ppm	6098386
Correlation Coefficient	**0.998**		**0.998**		**0.998**	

Table no.2: Precision data of the proposed HPLC method

Injection	Area for Hydrochlorothiazide	Area for Amlodipine	Area for Olmesartan
Injection-I	7001650	944170	4286742
Injection-II	7000180	944201	4287137
Injection-III	7001711	944153	4286253
Injection-IV	7001861	944108	4285164
Injection-V	7001234	944067	4284208
Average	7001327	944139	4285901
Standard Deviation	682.12	52.7	1200.8
%RSD	0.009	0.005	0.02

Table No. 3. Accuracy Studies

Drug	% Concentration	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	% Mean Recovery
Hydrochlorothiazide	50%	3583524	6.30	6.40	101.6%	100.9%
	100%	7001563	12.6	12.5	99.3%	
	150%	10078298	17.7	18.0	101.7%	
Amlodipine	50%	465931	2.5	2.47	99.07%	98.9%
	100%	935201	5.0	4.96	99.3%	
	150%	1387547	7.5	7.37	98.3%	
Olmesartan	50%	2182012	10.0	10.0	100.7%	98.9%
	100%	4285508	20.0	19.7	98.9%	
	150%	6112231	28.7	28.2	98.3%	

Table No.4 Assay Results

Formulation	Label Claim(mg)	Amount found (mg)	%Amount found
Formulation-I	12.5mg Hydrochlorothiazide, 5mg Amlodipine & 20mg Olmesartan	12.46mg Hydrochlorothiazide, 4.97mg Amlodipine & 19.64mg Olmesartan	99.7% Hydrochlorothiazide, 99.4% Amlodipine & 98.2% Olmesartan

Estimation of Hydrochlorothiazide, Amlodipine & Olmesartan in tablet dosage forms: Commercial formulation of Hydrochlorothiazide, Amlodipine & Olmesartan tablets were chosen for testing the suitability of the proposed method to estimate Hydrochlorothiazide, Amlodipine & Olmesartan in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 190 mg of Hydrochlorothiazide, Amlodipine & Olmesartan was transferred into a 10 mL volumetric flask and dissolved in 5 mL of a 40:60 v/v mixture of TEA buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 3 mL of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µm membrane filter. This solution of Hydrochlorothiazide, Amlodipine & Olmesartan was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table no.4.

RESULTS AND DISCUSSION

In the proposed method, the retention time of Hydrochlorothiazide, Amlodipine & Olmesartan was found to be 3.034 min., 4.062 min. & 5.165 min. respectively. Quantification was linear in the concentration range of in the range of 25 to 62.5ppm for the drug Hydrochlorothiazide & 10 to25ppm for the drug Amlodipine & 10 to100 ppm for the drug Olmesartan. The regression equation of the linearity plot of concentration of Hydrochlorothiazide, Amlodipine & Olmesartan over its peak area was found to be $Y = 2E+06x + 2E+06$ ($R^2 = 0.998$) for Hydrochlorothiazide, $Y= 22651x + 23756$ ($R^2 = 0.998$) for Amlodipine & $Y = 98624x + 1E+06$ ($R^2 = 0.998$) where x is the concentration of Hydrochlorothiazide, Amlodipine & Olmesartan (ppm) and y is the corresponding peak area. The number of theoretical plates calculated was 2876.9 for Hydrochlorothiazide, 3741.4 for Amlodipine & 3831.1 for Olmesartan which indicates efficient performance of the column. The LOD for the drugs Hydrochlorothiazide, Amlodipine & Olmesartan were found to be 0.009µg/ml**,** 0.06µg/ml & 0.06µg/ml respectively. The LOQ for the drugs Hydrochlorothiazide, Amlodipine & Olmesartan were found to be 0.03µg/ml**,** 0.2µg/ml & 0.2µg/ml respectively, which indicate the sensitivity of the method. The use of TEA buffer [pH 3.5] and acetonitrile in the ratio of 40:60 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug Hydrochlorothiazide, Amlodipine & Olmesartan by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, accurate and precise for the determination of Hydrochlorothiazide, Amlodipine & Olmesartan and can be reliably adopted for routine quality control analysis of Hydrochlorothiazide, Amlodipine & Olmesartan in its tablet dosage forms.

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