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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE HYDROCHLORIDE, AMLODIPINE BESYLATE AND OLMESARTAN MEDOXOMIL IN BULK AND COMBINED TABLETS DOSAGE FORMS

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ABSTRACT

A simple, selective, accurate high Performance Liquid Chromatographic (HPLC) method was developed and validated for the analysis of Chlorthalidone HCl, Amlodipine Besylate, and Olmesartan Medoxomil. The separation was achieved using Prontosil C_{18} column (250 x 4.6 mm, 5µm) and mobile phase consisting Buffer and Acetonitrile in the ratio 40: 60 with pH adjusted to 3.0 with orthophosphoric acid at flow rate of 1 ml/min. The retention time of Chlorthalidone HCl, Amlodipine Besylate and Olmesartan Medoxomil was found to be 3.017 mins, 3.313 mins and 5.010 mins respectively. The calibration curve were linear over the concentration ranges 26.75-38 µg/ml, 7.5-20 µg/ml and 30-80 µg/ml for Chlorthalidone HCl, Amlodipine Besylate, Olmesartan Medoxomil respectively. The proposed method is accurate with 99% recovery for Chlorthalidone Hydrochloride, 100.20% for Amlodipine Besylate and 98.97% recovery for Olmesartan Medoxomil in pharmaceutical preparation were all greater than 98% and their relative standard deviations were not more than 2.0%. Therefore, proposed method can be used as a more convenient and efficient option for the analysis of all drugs in bulk and tablet dosage form.

KEYWORDS: Chlorthalidone Hydrochloride, Amlodipine Besylate, Olmesartan Medoxomil, RP-HPLC, Validation.

INTRODUCTION

Hypertension is most commonly co-occurring cardiovascular risk factors. Coronary artery disease (CAD) is the leading cause of morbidity and mortality worldwide. It is a multifactorial disease, emphasis is to treat overall cardiovascular risk, rather than single risk factors in isolation.[1-2]

Chlorthalidone (CHL) is a sulphamyl benzophenone derivative, chemically known as [2-chloro-5-(1-hydroxyl-3-oxo-2, 3-dihydro-1Hisoindol-1-yl) benzene-1-sulfonamide]. It has a molecular formula of $C_{14}H_{11}ClN_2O_4S$ and molecular weight of 338.762 g/mol. It is considered a thiazide-like diuretic. It is used as an antihypertensive agent, diuretic and sodium chloride symporter inhibitor.[3]

Amlodipine Besylate is chemically 3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(o-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate. It has a molecular formula of $C_{26}H_{31}ClN_2O_8S$ and molecular weight of 567.05 g/mol. Amlodipine Besylate is a long-acting dihydropyridine calcium channel blocker. It is effective in the treatment of Angina Pectoris and Hypertension.[4]

Olmesartan Medoxomil (OMX) is (5-methyl-2-oxo-1, 3-dioxol-4-yl) methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] imidazole-4-carboxylate. It has a molecular formula of $C_{29}H_{30}N_6O_6$ and molecular weight of 558.595 g/mol Olmesartan Medoxomil is a synthetic imidazole derivative prodrug with an antihypertensive property. Olmesartan medoxomil is an Angiotensin II Type 1 Receptor Blocker that is used to manage hypertension.[5]

Chlorthalidone HCl, Amlodipine Besylate, and Olmesartan Medoxomil are available in the market as combined tablet dosage form, which is widely used in the treatment of hyper-tension and Angina pectoris.[3-5] The literature survey showed a very few chromatographic methods[6-10] were developed for the determination of Chlorthalidone HCl, Amlodipine Besylate, Olmesartan Medoxomil in a combination of either two drug. Thus, there's no method reported for simultaneous estimation of CHLOR, AML and OLM from dosage forms by RP-HPLC. The proposed method is optimized and validated as per the international conference on harmonization (ICH) guidelines.[11]

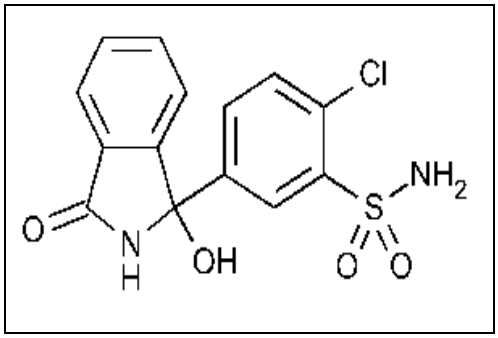

Figure 1: Structure of Chlorthalidone.

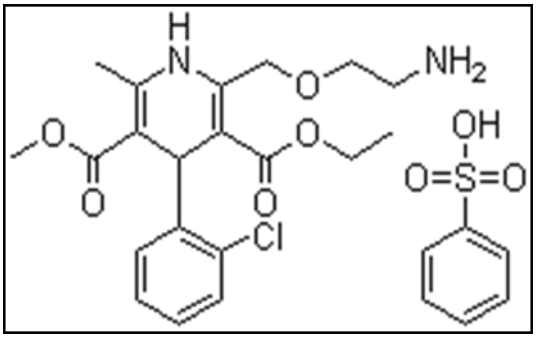

Figure 2: Structure of Amlodipine Besylate.

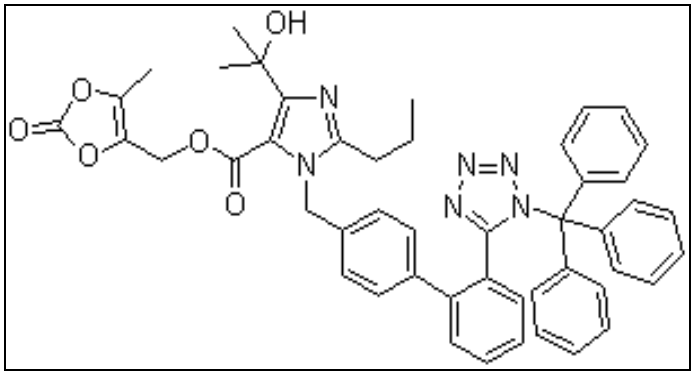

Figure 3: Structure of Olmesartan Medoxomil.

EXPERIMENTAL

Materials and reagents

Chlorthalidone HCl, Amlodipine Besylate, Olmesartan Medoxomil was obtained as gift sample from Euticals S.P.A, Prudence Pharma, Glenmark, Mumbai respectively. A commercial preparation (TRI OLMESAR CH 20 TABLET) used for analysis was procured from pharma market. Each tablet contains 12.5 mg of Chlorthalidone HCl, 5 mg of Amlodipine Besylate and 20 mg of Olmesartan Medoxomil, HPLC grade acetonitrile (Thomas Baker) and water, Potassium dihydrogen phosphate (LOBA CHEM), Orthophosphoric Acid.

Instrumentation

RP-HPLC was performed using Shimadzu HPLC system consisting of a pump LC-20AD, rheodyne sample injection port with 20 microlitre loop, SPD-20A UV-Detector, Spinchrom software, column used was Prontosil C_{18} (250 x 4.6 mm, 5 µm), Weighing was done on Contech CA-123 balance and pH was adjusted using PCI analytics Digital pH meter 111.

Principle

Reversed phase liquid chromatography isocratic elution with SPD-20A UV detection.

Chromatographic Conditions

Column	:	Prontosil C_{18} (250 x 4.6 mm, 5 µm)
Mobile Phase	:	Acetonitrile: 0.02 M Potassium dihydrogen phosphate buffer (60:40), adjusted to pH 3.0 with Orthophosphoric acid
Flow Rate	:	1.0 mL/min
Wavelength	:	240 nm
Injection Volume	:	20 µL
Runtime	:	10 minutes
Elution	:	Isocratic

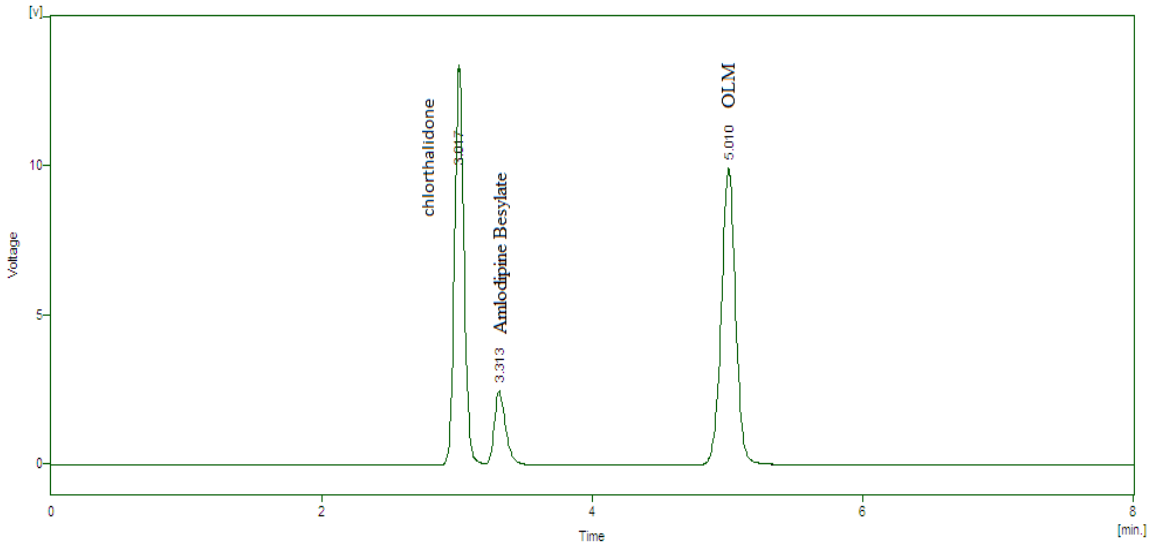

Figure 4: Chromatogram of Standard Solution.

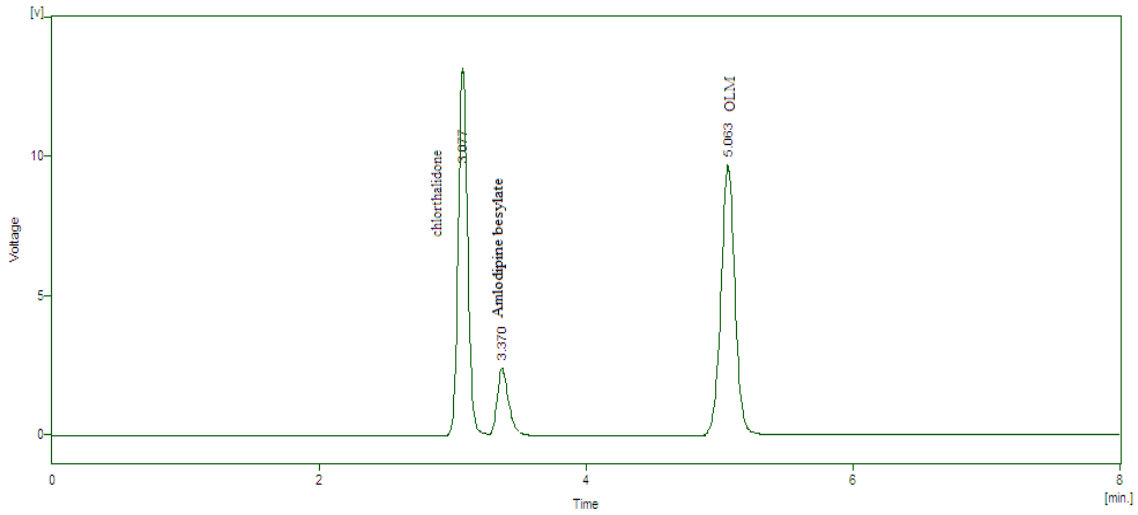

Figure 5: Chromatogram of Sample Solution.

Preparation of 0.02 M Potassium dihydrogen orthophosphate (pH 3.0)

About 2.7218 g of Potassium dihydrogen orthophosphate was accurately weighed and dissolved in 1000 ml of water and adjusted the pH with o-phosphoric acid to 3.0 ± 0.05. The solution was then filtered using 0.45 µ membrane filter.

Preparation of Mobile Phase

The pH of (0.02 M) Potassium dihydrogen orthophosphate was adjusted to 3.0 with Orthophosphoric acid, and mixed with Acetonitrile in the ratio 40:60 and was sonicated.

Preparation of Standard Solution

100 mg of Olmesartan Medoxomil, 100 mg of Chlorthalidone and 100 mg of Amlodipine Besylate standard were accurately weighed and transferred into 100 ml volumetric flask respectively. About 70 ml of mobile phase was added, sonicated to dissolve and diluted to 100 ml using mobile phase. Final concentration of Chlorthalidone HCl, Amlodipine Besylate, and Olmesartan Medoxomil were made to 50 µg/ml and 31.25 µg/ml and 12.5 µg/ml respectively by suitable dilutions.

Preparation of Sample solution

10 tablets were weighed and powdered. The quantity of powder equivalent to 20 mg Olmesartan Medoxomil, 12.5 mg of Chlorthalidone and 5 mg of Amlodipine Besylate were transferred into a 100 ml volumetric flask. About 70 ml mobile was added and solution was sonicated for 30mins with intermittent shaking. The volume was made up using the mobile phase, mixed and filtered through 0.45µ PVDF filter.Final concentration of Chlorthalidone HCl, Amlodipine Besylate, Olmesartan Medoxomil were made to 50 µg/ml and 31.25 µg/ml and 12.5 µg/ml respectively by suitable dilutions.

RESULT AND DISCUSSION

The proposed RP-HPLC method was validated as per ICH guidelines.

Selectivity and Specificity

To assess the selectivity of the developed method solutions of all three drugs were injected into the system then observe three sharp peaks of Chlorthalidone HCl, Amlodipine Besylate and Olmesartan Medoxomil were obtained at retention time of 3.077 mins, 3.370 mins and 5.063 mins respectively in reference to standard solution. Specificity was determined by comparison of the chromatogram of mixed standards and sample solutions. As the retention

time of standard drugs and the retention time of the drugs in sample solutions were same, so the method was specific. The parameters like resolution (Rs) and asymmetric factor were calculated. Good correlation was found between the results of mixed standards and sample solutions. Results are shown in the Table 1.

Linearity

The linearity of an analytical method is its ability to obtain results, which are directly proportional to the concentration of analyte in the sample. It was carried out by preparing the sample solutions containing 26.75-38 µg /ml, 7.5-20 µg/ml, 30-80 µg/ml Chlorthalidone HCl, Amlodipine Besylate and Olmesartan Medoxomil respectively. A calibration curve was drawn by plotting concentration on X-axis Vs area on Y-axis and regression equation, correlation coefficient, y-intercept, slope of the equation was calculated. Result are shown in the Table 2 and Figure 6, 7, 8.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 75 %, 100 % and 125 %. The recovery studies were carried out by adding known amounts of standard Chlorthalidone HCl, Amlodipine Besylate and Olmesartan Medoxomil were added to pre-analyzed samples and they were subjected to proposed HPLC method. The recoveries results of standards in pharmaceutical preparation are shown in the Table 3.

Precision

Precision study was performed to find out intraday and interday variations. The intraday and interday precision study of Chlorthalidone HCl, Amlodipine Besylate and Olmesartan Medoxomil was carried out by estimating the correspondence response 3 times on the same day and on 3 different days for 3 different concentrations and the results are reported in terms of % relative standard deviation (% RSD) however, all results fall within acceptance limits (RSD < 2), as shown in Table 4.

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

LOD is ability of analytical method able to detect the lowest concentration of the analyte. LOQ is lowest concentration of the analyte which can be quantitatively analyzed with acceptable precision and accuracy. It was calculated based on the slope and blank response from the calibration curve as per ICH guidelines. LOD and LOQ were calculated based on the standard deviation of the response and slope. Result are shown in Table 4.

Robustness

The robustness study was done by making small changes in the optimized method parameters like ± 2 % change in flow rate, ± 2 % change in mobile phase ratio, and ± 2 % change in wavelength. There was no significant impact on the retention time and tailing factor.

Assay

The amount of Chlorthalidone HCl, Amlodipine Besylate and Olmesartan Medoxomil per tablet was calculated by comparing the peak area of the standard solution and sample. Result are shown in Table 5.

Table 1: System suitability parameters.

System Suitability Parameters	CHLOR	AMLO	OLM
Retention time (min)	3.07	3.370	5.063
Resolution	2.91	2.33	6.81
Theoretical plates	8653	7524	10203
Asymmetric factor	1.10	1.57	1.063

Table 2: Linearity studies.

PARAMETERS	CHLOR	AMLO	OLM
Linearity range	26.75-38 µg /ml	7.5-20 µg/ml	30-80 µg/ml
Slope	38.445	21.686	25.606
Intercept	5.253	1.938	2.486
Correlation coefficient	0.999	0.9998	0.9998

Linearity Data

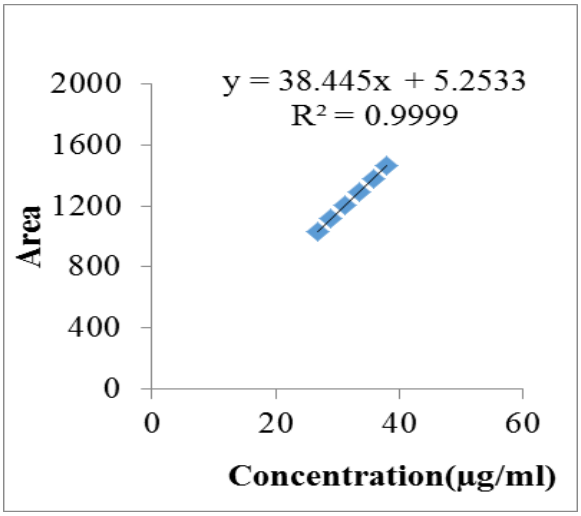

Figure 6: Calibration curve of Chlorthalidone HCl.

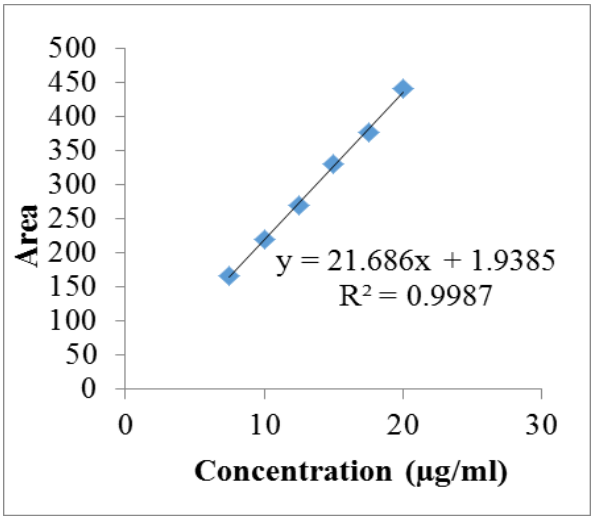

Figure 7: Calibration Curve of Amlodipine Besylate.

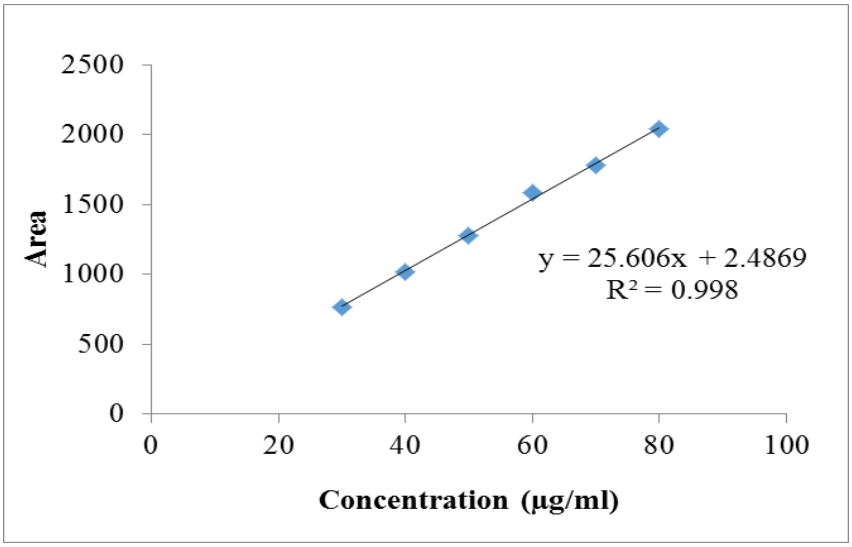

Figure 8: Calibration Curve of Olmesartan Medoxomil.

Table 3: Results of Accuracy studies.

Pre-analyzed sample solution [µg/ml]	Sample concentration [µg/ml]	Excess drug added [µg/ml]	Amount recovered [µg/ml]	% Recovery
CHLOR	15.63	7.812	23.221	98.06%
	15.63	15.63	30.99	98.14
	15.63	23.43	39.23	99.43
AMLO	6.25	3.13	9.26	97.73
	6.25	6.25	12.40	98.20
	6.25	9.38	15.52	98.30
OLM	25	12.5	37.38	98.68
	25	25	49.92	98.84
	25	37.5	62.32	98.71

Table 4: Results of precision and LOD & LOQ

Parameters	CHLOR	AMLO	OLM
	Precision (% RSD)		
Intra-day (n=3)	0.87	0.43	0.52
Inter-day (n=3)	0.67	0.87	0.59
Limit of detection	0.57	1.86	9.32
Limit of quantitation	0.18	0.61	3.07

Table 5: Assay Determination of Chlorthalidone HCl, Amlodipine Besylate, Olmesartan edoxomil.

Brand		% Amount Found
TRI OLMESAR CH (12.5 mg CHLOR + 5 mg AMLO + 20 mg OLM)	**CHLOR**	99.00
	AMLO	100.20
	OLM	98.97

CONCLUSION

The developed method is the first report for simultaneous estimation of Chlorthalidone Hydrochloride, Amlodipine Besylate and Olmesartan Medoxomil. The developed HPLC method was found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of this method showed good results.

The recovery studies revealed excellent accuracy and high precision of the method. The statistical parameters and recovery studies of HPLC method were compared with the developed spectrophotometric method of analysis of the same dosage form. The HPLC method was found to give better results. Therefore the proposed method could be applied for routine analysis in quality control laboratories.

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