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QUANTIFICATION AND VALIDATION OF AMLODIPINE BESYLATE, OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE BY RP-HPLC IN MARKETED DOSAGE FORM

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Keywords:

Amlodipine, Hydrochlorothiazide, Olmesartan medoxomil, RP-HPLC, Validation

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ABSTRACT: The endeavor of the present work is to develop a simple, economical, efficient, novel green analytical method for the estimation of Amlodipine besylate, Olmesartan medoxomil and Hydrochlorothiazide in pharmaceutical formulation. Quantification was carried out using an Inertsil CN-3.5 µm (4.6 ×250 mm) column, where the mobile phase consisting of 10 mm Phosphate buffer (pH 3.0) and Acetonitrile (40:60). The flow rate was 1.0 mL/min and the effluent was monitored at 262 nm. The observed linearity was in the range of 5-25 µg/ml for Amlodipine (AMLO), Hydrochlorothiazide (HCTZ) and Olmesartan medoxomil (OLME) with a correlation coefficient of 0.997, 0.999 and 0.999 respectively. The proposed method was validated as per ICH guidelines in terms of linearity, accuracy, precision, robustness, and specificity, the limit of detection and limit of quantification. The method has been applied to Amlodipine, Hydrochlorothiazide and Olmesartan formulation without the interference of excipients of the formulation.

INTRODUCTION:

Amlodipine Besylate: Amlodipine Besylate (2-[(2-Aminoethoxy) methyl]- 4- (2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester benzene sulfonate). Amlodipine is an L-type calcium channel blocker, which decreases the contraction of action and myosin fibers in the cardiac tissue by decreasing the supply of calcium ions. This results in a significant decrease in blood pressure [1, 4-8]. The chemical structure was shown in **Fig. 1**.

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Formula: $C_{26}H_{31}ClN_2O_8S$

Chemical Structures of Drugs:

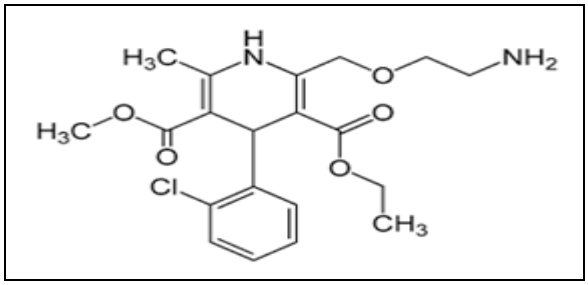

FIG. 1: CHEMICAL STRUCTURE OF AMLODIPINE BESYLATE

Hydrochlorothiazide: Hydrochlorothiazide (6-chloro- 1, 1- dioxo- 3, 4- dihydro- 2H- 1, 2, 4-benzothiadiazine-7-sulfonamide) is a thiazide-type diuretic, which causes an increased elimination of fluid in the urine, thereby decreasing the blood

volume, resulting in a decrease in blood pressure [2, 4-8]. The chemical structure was shown in **Fig. 2.**

Formula: $C_7H_8C_lN_3O_4S_2$

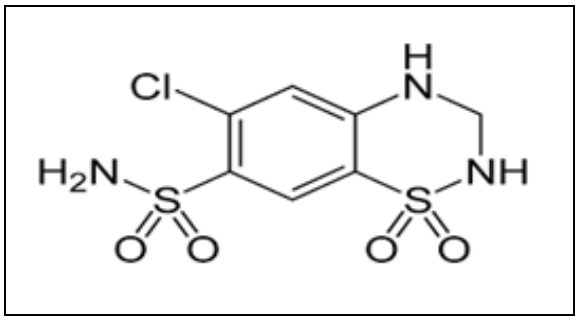

FIG. 2: CHEMICAL STRUCTURE OF HYDRO-CHLOROTHIAZIDE

Olmesartan Medoxomil: Olmesartan Medoxomil 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 is an Angiotensin-converting enzyme II inhibitor, which by inhibiting that receptor, breaks the Renin-Angiotensin system cycle that usually regulates (increases blood pressure based on mineral content in blood) blood pressure [3-8]. Thus, it inhibits an increase in blood pressure. The chemical structure was shown in **Fig. 3**.

Formula: $C_{29}H_{30}N_6O_6$

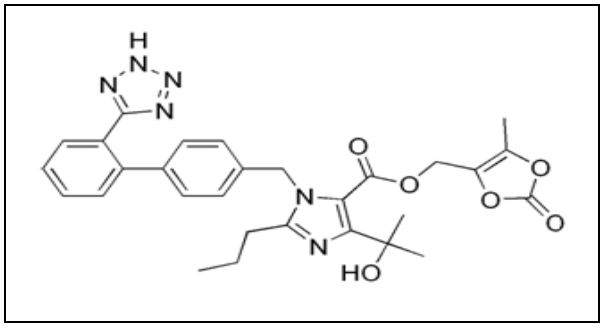

FIG. 3: CHEMICAL STRUCTURE OF OLMESARTAN MEDOXOMIL

MATERIALS AND METHODS:

Chemicals and Reagents: [9-10] Aurobindo Pharma Pvt Ltd, Hyderabad, India kindly supplied the pure working standards of the known potency of AMLO, HCTZ and OLME as a gift sample. The marketed sample with the strength of OLME (20 mg), AMLO (5 mg) and HCTZ (12.5 mg) manufactured and marketed by Macleods Pharmaceuticals Ltd, purchased from the local Pharmacy. The reagents like Orthophosphoric Acid (OPA) of Hi-Media Laboratories Pvt. Ltd, Water, Acetonitrile, Triethylamine of Merk, Potassium dihydrogen phosphate of Thermo Fisher Scientific India Pvt. Ltd where used.

Instrumentation: The HPLC system (Agilent HPLC 1200 Infinity LC Specifications) consisted of a pump (Agilent LC20AT) programmed with Ezchrom Elite Software and rheodyne Injector was used. The detector consisted of a UV/VIS (UV-2489) model that was operated at a wavelength of 262 nm. The column used was Inertsil CN- 3 columns at ambient temperature [11-15].

Preparation of Standard Stock Solution: Accurately weighed Olmesartan Medoxmil (20 mg), Amlodipine (5 Mg) And Hydrochlorothiazide (12.5 mg) were transferred to 100 ml volumetric flask and dissolved and diluted to the mark with methanol. The stock solution further diluted with methanol to obtain a solution of OLME (20 µg/ml), AMLO (5 µg/ml), and HCTZ (12.5 µg/ml), respectively [20-24].

Optimization of HPLC Method: [16-19] The HPLC procedure was optimized with a view to developing a simultaneous assay method for OLME, AMLO and HCTZ respectively.

The mixed standard stock solution (20 µg/ml for OLME and 5 µg/ml for AMLO and 12.5 µg/ml for HCTZ) was injected. For HPLC method optimization of different ratios of methanol and water were tried but it was found that 10 mM Phosphate buffer (pH 3.0): Acetonitrile (40:60) adjusted with OPA gives acceptable retention time (Rt), plates and good resolution for OLME, AMLO, and HCTZ.

TABLE 1: INSTRUMENTATION OF HPLC

Specifications	
Software	Ezchrom Elite
Column	Inertsil CN-3.5µm (4.6 x 250 mm)
Pump	Agilent LC20AT
Detector	UV/VIS (UV-2489)
Injector	Rheodyne
Temperature	Ambient

Method Validation as per ICH: [4-5, 25] After the development of the RP-HPLC method for the estimation of the drug in a dosage form, validation of the method was performed. This part describes the procedure followed for the validation of the developed method.

TABLE 2: DETAILS OF FORMULATION

Brand Name	Manufactured By	Concentration of Olmesartan Medoxomil (mg)	Concentration of Amlodipine Besylate (mg)	Concentration of Hydrochlorothiazide (mg)
Tri Olmesar 20	Macleods Pharmaceuticals Ltd	20	5	12.5

Linearity: A series of standard solutions (not less than 5 is recommended) were prepared in the range of 5 µg/ml- 25 µg/ml containing Amlodipine, Hydrochlorothiazide and Olmesartan standards and injected. A plot of average peak area versus the concentration in µg/ml or mg/ml is made and from this the correlation coefficient, y-intercept (constant of regression) and slope (coefficient of regression) of the regression line were calculated. The calibration data of Amlodipine, Hydrochlorothiazide and Olmesartan is given in **Table 3** and the calibration curve is shown in **Fig. 4, 5** and **6**.

TABLE 3: TABLE OF RESULTS OF LINEARITY OF DETECTOR RESPONSE

Standard conc. (µg/ml)	Area			Mean Area		
	Amlodipine	Hydrochlorthiazide	Olmesartan	Amlodipine	Hydrochlorothiazide	Olmesartan Medoxomil
10	787048	4398140	3686014			
	787132	4398319	3686166	787090	4398229.5	3686090
20	1488335	9480590	7783039			
	1488246	9480649	7783165	1488290.5	9480619.5	7783102
45	2065785	13932234	11477968			
	2065891	13932378	11477863	2065838	13932306	11477915.5
80	2958656	18941459	14965826			
	2958633	18941229	14965647	2958644.5	18941344	14965736.5
160	3519305	24174846	19466729			
	3519210	24174766	19466738	3519257.5	24174806	19466733.5

Regression Amodipine = 0.997 Hydrochlorthiazide = 0.999 Olmesartan = 0.999

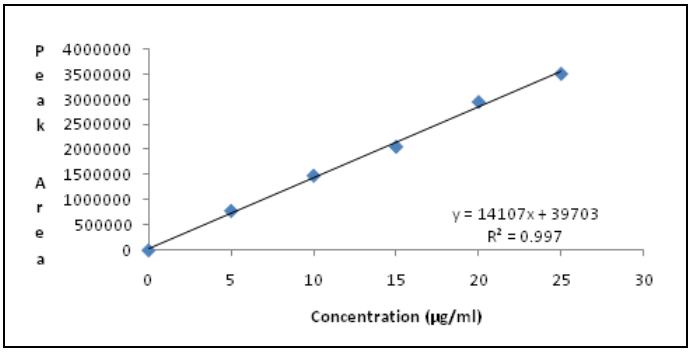

FIG. 4: LINEARITY PLOT OF AMLODIPINE BESYLATE

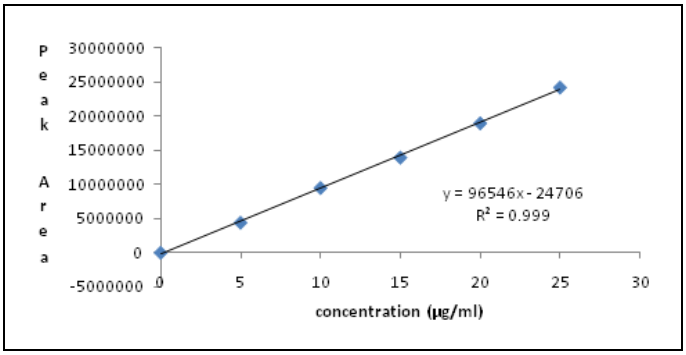

FIG. 5: LINEARITY PLOT OF HYDROCHLOROTHIAZIDE

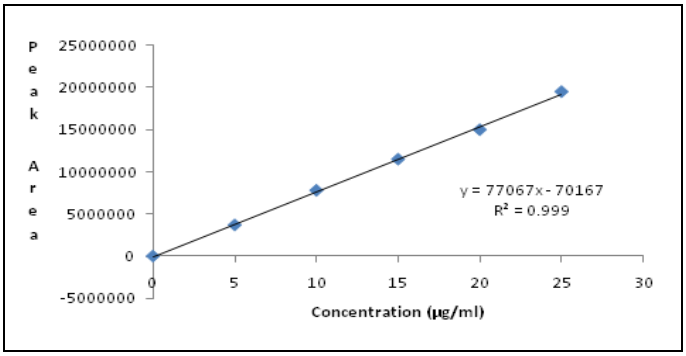

FIG. 6: LINEARITY PLOT OF OLMESARTAN MEDOXOMIL

Precision: The precision of the test procedure was evaluated by injecting the six standard solutions. The relative standard deviation of six injections was calculated. The result of Precision studies is given in **Table 4**.

TABLE 4: PRECISION FOR AMLODIPINE, HYDROCHLOROTHIAZIDE AND OLMESARTAN MEDOXOMIL

S. no.	Injection Number	Peak area for Amlodipine	Peak area for Hydrochlorothiazide	Peak area for Olmesartan
1	Standard 1	2061785	13982634	11487359
2	Standard 2	2066391	13993376	11477765
3	Standard 3	2068990	13925439	11426629
4	Standard 4	2063526	13926128	11399957
5	Standard 5	2065891	13892034	11417556
6	Standard 6	2057643	13932431	11458733
	Mean	2064037.67	13942007.00	11444666.50
	%RSD	0.18	0.25	0.28

Specificity: Specificity is the ability of a method to discriminate between the analyte (s) of interest and other components that are present in the sample. A study of placebo interference from excipients was conducted. An equivalent weight of placebo taken as per the test method and placebo interference was conducted in duplicate.

Accuracy: To validate whether the test method can accurately quantify Amlodipine, Hydro-chloro-thiazide, and Olmesartan, prepare samples in three times for higher and lower levels, in triplicate for other levels by spiking Amlodipine, Hydro-chlorothiazide and Olmesartan active material with an equivalent amount of placebo and perform CU as per test procedure. Samples were prepared at levels 50%, 100% and 150% of the target assay concentration *i.e.* 50% of the lowest strength initial concentration to 150% of the highest strength initial concentration level. **Table 5** shows the results for the accuracy of Amlodipine, Hydro-chlorothiazide and Olmesartan Medoxomil.

TABLE 5: ACCURACY FOR AMLODIPINE, HYDROCHLOROTHIAZIDE AND OLMESARTAN

% Spiked	Weight added (mg)			Weight recovered (mg)			% Recovery		
	Amlo-dipine	Hydrochlor othiazide	Olmesarta n	Amlo-dipine	Hydrochl-orothiazide	Olmesarta n	Amlo-dipine	Hydroch-lorothiazide	Olmesartan
50	2.50	6.26	10.29	2.46	6.33	9.912	98.40	101.12	96.32
	2.50	6.38	10.25	2.48	6.29	9.910	99.20	98.59	96.68
	2.52	6.23	10.22	2.49	6.46	9.908	98.81	103.69	96.95
100	5.08	12.54	20.07	4.88	12.47	19.45	96.06	99.44	96.91
	5.12	12.56	20.13	4.98	12.79	19.42	97.26	101.83	96.47
	5.06	12.58	20.04	4.76	12.29	19.40	97.07	97.69	96.81
150	7.57	18.89	30.04	7.39	18.79	29.37	97.62	99.47	97.78
	7.73	18.73	30.03	7.68	18.83	29.33	99.35	100.53	97.67
	7.58	18.71	30.07	7.47	18.66	29.32	98.55	99.73	97.51

Assay: The amounts OLME, AMLO and HCTZ per tablet were calculated by extrapolating the value of area from the calibration curve. The analysis procedure was repeated six times with tablet formulation. The result formulation was reported in **Table 6**.

TABLE 6: RESULTS OF %ASSAY OF AMLODIPINE, HYDROCHLOROTHIAZIDE AND OLMESARTAN MEDOXOMIL

Name	As	At	Wt. equivalent taken (mg)	% Assay
Amlodipine	787048	787012	5.0	99.495
Hydrochlorthiazide	11706412	11706368	12.5	98.402
Olmesartan Medoxomil	14965826	14965799	20	96.499

Summary: [4, 5]

TABLE 7: SUMMARY OF VALIDATION PARAMETERS BY HPLC METHOD

Validation	Parameters	Amlodipine	Hydrochlorothiazide	Olmesartan Medoxomil
System suitability	Tailing factor	1.16	1.10	1.08
	% RSD	0.59	0.39	0.29
	Theoretical plates	7954	10346	9610
	Resolution	N.A	5.55	5.44
Linearity	Correlation coefficient	0.997	0.999	0.999
	Slope	14107	96546	77067

Precision	% RSD	0.18	0.25	0.28
Accuracy	Mean % recovery for 50, 100, 150% respectively	98.80 96.80 98.50	102.80 99.65 99.91	96.65 96.73 97.65
Specificity	Interference	No interference	No interference	No interference
Robustness	Flow rate by ± 10% Column Oven temperature by ± 5 °C pH of Buffer solution by ± 0.2 units Wavelength of analysis ± 5 nm Organic composition of mobile phase by ± 5%	All the All the system suitability parameters are within the limit for all the variable parameters, for all the three drugs		
LOD	Standard deviation	0.85 µg/ml	1.20 µg/ml	1.37 µg/ml
LOQ	method	2.58 µg/ml	3.64 µg/ml	4.16 µg/ml

CONCLUSION: HPLC method was developed and validated as per ICH guidelines. It can be concluded that the method is specific for the estimation of OLME, AMLO, and HCTZ in the pharmaceutical dosage form. The method has a linear response in a stated range and is accurate and precise.

Statistical analysis proves that the method is suitable for the analysis of OLME, AMLO, and HCTZ as bulk drug and in the pharmaceutical formulation without any interference from the excipients [26-39].

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CONFLICTS OF INTEREST: The authors confirm that this article content has no conflict of interest.

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