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HPLC method development and validation for simultaneous estimation of Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Besylate tablets

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ABSTRACT

In the present work for the determination of Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine a simple, rapid and precise high performance liquid chromatography procedure has been developed and validated. The separation and quantification were achieved on a Cyano C18 column using a mobile phase of filtered and degassed mixture of buffer and acetonitrile in the ratio of 830:170 with of analytes at 237 nm. Buffer was prepared by dissolving 3.65 g of ammonium acetate in 1000 ml of water and the pH was adjusted to 7.0 with sodium hydroxide at flow rate of 1.5 ml/min. The method showed good linearity with different concentrations near to 0.99909, 0.99902 and 0.99871 for Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine respectively. The recovery (Average mean & RSD) were achieved as 102.18, 1.01 for Olmesartan Medoxomil, 102.58, 0.26 for Hydrochlorothiazide and 101.37, 0.26 for Amlodipine respectively. The precision (Average mean & RSD) were achieved as 99.9, 104.02 and 99.80 for Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine respectively.

Keywords: HPLC, Amlodipine Besylate, Olmesartan Medoxomil, Hydrochlorothiazide.

INTRODUCTION

The total number of drugs released in to the market is increasing dramatically over the past decades across the globe. Most of the drugs are either the partial structural modification of the existing drugs or new entities [1]. In some cases there will be time lag from the date of drug introduction in to the market to the date of its inclusion in pharmacopoeias. This usually happens as a result of uncertainity in continuous wider usage of these drugs, toxicity reports, development of resistance with already existing methods, or it may not provide enough adequate sensitivity. In these conditions, sufficient standard analytical procedures may not be available in the pharmacopoeias due to patent regulations and moreover there may not be suitable method for a particular analyte in the specific sample matrix [2-3]. Therefore it becomes necessary to develop new analytical methods for such drugs. Methods for drug analysis can be developed provided one should possess sound knowledge about the nature of the sample with respect to its polarity, molecular weight, solubility parameter and ionic character. Now a day the development of method for drug analysis is usually based on prior art or existing literature using the same quite similar instrumentation. It is very rare that an HPLC method is developed that does not in some way relate to existing literature-based methods. The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte as well as to the final requirements of the method.

HPLC is considered to be most suitable analytical method for pharmaceuticals and remains as useful tool in future despite further advances in analytical chemistry because of several overhelming advantages for the solution of many problems. These advantages include speed, simplicity, specificity and sensitivity. Literature studies reveal that there are analytical methods available for determination of Amlodipine, Olmesartan and Hydrochlorothiazide in combination with other drugs [4-18] and only few analytical methods available for simultaneous estimation of Amlodipine, Olmesartan and Hydrochlorothiazide in combined form. Doshi *et al.*, have reported validated reverse phase HPLC method for simultaneous estimation of all the three drugs by using Buffer:Acetonitrile (containing 10% Tetrahydrofuran) as mobile phase [19]. Nalluri *et al.*, have reported development and validation of reverse phase HPLC-PDA method for simultaneous estimation of Amlodipine, Olmesartan and Hydrochlorothiazide in combined form by using 0.1% v/v formicacid:acetonitrile as mobile phase [20]. Although the methods shows optimum retention time it fails to provide detailed explanation on stability control and lacks validation in terms of stress conditions like acid, base, heat, oxidation, light and UV. Also the peak shapes and resolution of the above two methods were also not appropriate. Kumar *et al.*, have reported ultra performance liquid chromatography method development for simultaneous estimation of all the three drugs by using sodium perchlorate:acetonitrile as mobile phase [21]. This method does not provide complete validation of all the three drugs in terms of its system suitability and robustness. Moreover the method developed was not completely reproducible and the peak resolution was not good. Hence in the present work it was planned to develop a stability indicating HPLC method, which would be precise, accurate, simple, reproducible and reliable for marketed drug combination of Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Besylate tablets. The objective thus, was to subject this new tablet formulation to HPLC analysis by different trial methods and finally arrive at a suitable method for the determination of the drugs therein. The present work was mainly confined to the estimation of content of active ingredient with good resolution and optimum retention time simultaneously covering the validation assays completely as per ICH guidelines [22-23].

Olmesartan Medoxomil belongs to the class of medicines called angiotensin II receptor antagonists to treat high blood pressure and available in 5, 20 and 40 mg tablet. Olmesartan is a prodrug having one tetrazole and one imdazole group. It usually blocks the binding of angiotensin-II to the AT_1 receptors in vascular muscle thereby reduces vasoconstriction and secretion of aldosterone which automatically lowers blood pressure by producing vasodilation and decreasing peripheral resistant [24]. Amlodipine Besylate is a long-acting calcium channel blocker generally used as an anti-hypertensive and in the treatment of angina. It inhibits calcium ion influx across cell membrane selectively with more effect on smoother muscles than on cardiac muscles thereby reducing peripheral vascular resistant and blood pressure. Hydrochlorothiazide belongs to the thiazide class of diuretics, mainly acting on the kidneys to reduce sodium reabsorption in the distal convoluted tubule. It helps in the reabsorption of calcium in distal convoluted tubule simultaneously lowering peripheral vascular resistance [25].

MATERIALS AND METHODS

HPLC instrument model name: SPD-M10Avp (Shimadzu) PDA Detector. Software: Class VP.

Chemicals: Acetonitrile HPLC Grade (Qualigens pvt.ltd), Methanol (Qualigens pvt.ltd), Ammonium acetate AR (E-Merk Ltd,.India), Sodium hydroxide AR (E-Merk Ltd,.India), Milli-Q-water (Rankem) Reference standard purity: Olmesartan Medoxomil (99.92 %), Hydrochlorothiazide (100 %), Amlodipine Besylate (99.22 %). Each film coated tablet contains Olmesartan Medoxomil (20 mg), Hydrochlorothiazide (12.5 mg) BP, and Amlodipine Besylate 5 mg BP equivalent to Amlodipine (Micro Labs Ltd).

Analytical method development

Trail Method: 1

As per in-house method (product release specification) combination of Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorothizide.

Chromatographic conditions

Mobile phase composition:

Buffer: Acetonitrile: Methanol (70:10:30) [Buffer - 0.05 M Ammonium acetate in 1000 ml of water adjust the pH to 7.0 with NaOH.

Column	: Cyano C18, 250 × 4.6 mm, 5 µm (thermo hypersil column)
Detector	: 225 nm

Diluent : Acetonitrile : Water (80:20)
Flow rate : 1.5 ml/min
Injection volume : 10 µl
Concentration of standard: Olmesartan Medoxomil (0.1 mg/ml), Hydrochlorothiazide (0.0625 mg/ml) and Amlodipine (0.025 mg/ml)

Inference: Amlodipine peaks completely merged with Olmesartan peaks and peak resolution was not good. So attempt was taken to increase the difference in retention time by increasing the proportion of acetonitrile which resulted in peak broadening. Fig.1A, Table:1

Table 1

S.No	Peak Name	Retention Time (min)	Area (mAU X min)	Relative Area (%)	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1.	Hydrochlorothiazide	4.076	4768322	61.25	15.15	0.86	2756
2.	Olmesartan	11.375	2807118	36.06	0.00	1.09	4786
3.	Amlodipine	12.242	209157	2.69	1.42	1.25	7502
Total			7784596	100.00	16.57	3.20	15044.00

Trail Method: 2
Different mobile phase composition
Chromatographic conditions
Buffer: Acetonitrile: Methanol (60:20:20) [Buffer - 0.05M Ammonium acetate in 1000 ml of water] the pH was adjusted to 7.0 with NaOH
Column : Cyano C18, 250 × 4.6 mm, 5 µm (thermo hypersil column)
Detector : 225 nm
Diluent : Acetonitrile: Water (80:20)
Flow rate : 1.5 ml/min
Injection volume : 10 µl
Concentration of standard: Olmesartan Medoxomil (0.1 mg/ml), Hydrochlorothiazide (0.0625 mg/ml) and Amlodipine (0.025 mg/ml).
Inference: Peak resolution was not good but decreasing the volume of Buffer and methanol some extend resulted in broadening of peaks. Fig.1B, Table: 2.

Table 2

S.No	Peak Name	Retention Time (min)	Area (mAU X min)	Relative Area (%)	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1.	Hydrochlorothiazide	3.633	4702869	58.78	n.a	0.81	2942
2.	Olmesartan	5.592	2924008	36.54	n.a	1.13	4517
3.	Amlodipine	6.408	374574	4.68	n.a	1.18	5793
Total			8001451	100.00	0.00	3.12	13252.00

Trail Method: 3
All the chromatographic conditions remain same except the buffer:Acetonitrile:Methanol (50:40:10)
Inference: Peak broadening is observed. Fig.1C, Table: 3.

Table 3

S.No	Peak Name	Retention Time (min)	Area (mAU X min)	Relative Area (%)	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1.	Hydrochlorothiazide	3.417	4589096	58.75	6.99	0.94	4084
2.	Olmesartan	4.058	2836735	36.32	0.00	1.19	5642
3.	Amlodipine	4.767	385360	4.93	24.87	1.26	7152
Total			7811190	100.00	6.21	3.39	16878.00

Trail Method: 4
Chromatographic conditions
Mobile phase composition:

Buffer : Acetonitrile (87:13) [Buffer - 0.05 M Ammonium acetate in 1000 ml of water] the pH was adjusted to 7.0 with NaOH
Column : Cyano, 250 × 4.6 mm, 5 µm (thermo hypersil column)
Detector : 237 nm
Diluent : Acetonitrile : Water (80:20)
Flow rate : 1.5 ml/min
Injection volume : 10 µl

Concentration of standard: Olmesartan Medoxomil (0.1 mg/ml), Hydrochlorothiazide (0.0625 mg/ml) and Amlodipine (0.025 mg/ml)

Inference: The mobile phase composition gave good resolution and optimum retention time with appropriate tailing factor (< 2). The detected wave length was selected as 237.0 nm where the peaks height of the drug was acceptable. Fig.1D, Table: 4.

Table 4

S.No	Peak Name	Retention Time (min)	Area (mAU X min)	Relative Area (%)	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1.	Hydrochlorothiazide	3.042	256218	12.51	6.85	1.07	2785
2.	Olmesartan	5.792	1458068	71.17	0.00	0.90	1623
3.	Amlodipine	25.638	334565	16.33	25.67	1.12	3729
Total			2048850	100.00	32.52	3.09	8137.00

RESULTS AND DISCUSSION

Method of Analysis validated
Chromatographic system:
HPLC : Shimadzu HPLC with PDA or UV detector and Class VP software
Analytical Column : Cyano C18, 250 × 4.6 mm, 5 µm (thermo hypersil column)
Detector wave length : 237 nm
Diluent : Acetonitrile : Water (80:20)
Flow rate : 1.5 ml/min
Injection volume : 10 µl
Column temperature : Ambient

Buffer:
3.895 g of Ammonium Acetate was dissolved in 1000 ml of water and the pH was adjusted to 7.0 with NaOH solution and filtered.

Mobile Phase:
Buffer and Acetonitrile were taken in the ratio of 830:170, mixed well and degassed.

Diluent:
Acetonitrile and Water was taken in the ratio of 80:20
0.1 N HCl Preparation:
8.5 ml of Conc. HCl was added to about 500 ml of water mixed well and made up to 1000 ml with water.

Standard Preparation:
A weighed quantity of about 100 mg of Olmesartan Medoxomil working standard, 62.5 mg of Hydrochlorothiazide and 25 mg of Amlodipine Besylate was transferred accurately to a 100 ml volumetric flask to this 15 ml of 0.1 N HCl was added and kept in sonicator to dissolve the contents and made up to the volume with diluents. 5 ml of the above solution was diluted to 50 ml with diluent and the resultant solution was filtered through 0.45 µ membrane filter.

Sample Preparation:
About 20 tablets was crushed into fine powder, and equivalent tablet powder 100 mg of Olmesartan Medoxomil was weighed and taken into 100 ml volumetric flask to this 15 ml of 0.1 N HCl was added and kept in sonicator to dissolve the contents and made up to the volume with diluents. 5 ml of the above solution was diluted to 50 ml with diluent and the resultant solution was filtered through 0.45 µ membrane filter.

System suitability
The standard preparation was chromatographed and peak responses were recorded as directed under procedure. The theoretical plates for Olmesartan peak, Hydrochlorothiazide peak, Amlodipine peak should not be less than 2000, tailing factor should not be more than 2.0 and percent relative standard deviation for five replicate injections of standard preparation should not be more than 2.0 % .

Procedure
10 µl of standard preparation and sample preparation was injected seperately into the chromatograph and peak responses for major peaks were measured.

System Precision
System precision were performed daily during entire validation of the method by using following two methods.

Repeatability
The repeatability of the analytical method was established by estimating the assay for six different sample preparations of same batch. Assays for all the six sample preparations were calculated and their %RSD of 6 assays were tabulated. Theoretical plates obtained for Olmesartan, Hydrochlorothiazide and Amlodipine peaks are 2135, 2466 and 2308 respectively tabulated in Table: 5a

Intermediate Precision (Ruggedness)
The ruggedness of the analytical method was established by estimating the assay for six different sample preparations of same batch by a different analyst using a different HPLC system with different similar column on a different day. The test method meets the criterion for system precision and the results were tabulated in Table :5b

Selectivity
The selectivity of the method demonstrated by injecting the blank solution (mobile phase), Placebo preparation (Excipients), individual Olmesartan Medoxomil working standard (WS) preparation, Hydochlorothiazide WS preparation and Amlodipine Besylate WS preparation, sample preparation and mixture of Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Besylate WS solution. Table : 6a-6d

Table 5a System Precision- Repeatablity

Injection No	Area		
	Olmesartan	Hydrochlorothiazide	Amlodipine Besylate
1	1366054	364521	293853
2	1364054	368124	294517
3	1356571	366542	291587
4	1378017	363245	296844
5	1380172	364521	297685
Mean	1368974	365391	294897
SD[a]	9921	1931	2437
% RSD[b]	0.72	0.53	0.83

[a] Standard Deviation; [b] Relative Standard Deviation

Table 5b Intermediate Precision

Injection No	Area		
	Olmesartan	Hydrochlorothiazide	Amlodipine Besylate
1	1412076	569900	283720
2	1424234	578726	287822
3	1431572	578756	294196
4	1435995	574175	288987
5	1459726	586074	291868
Mean	1432721	577526	289319
SD[a]	17604	6029	3999
% RSD[b]	1.23	1.04	1.38

[a] Standard Deviation; [b] Relative Standard Deviation

Table 6a Drug product specificity- Olmesartan

Olmesartan		
Sample	% Assay	Peak purity
Unstressed	99.91	-
Acid	10.95	0.999982
Base	ND[a]	-
Oxidation	95.80	0.999907
Heat	98.40	0.999922
Light	92.40	0.999935
UV	95.65	0.999923

[a] No Data

Table 6b Drug product specificity- Hydrochlorothiazide

Hydrochlorothiazide		
Sample	% Assay	Peak purity
Unstressed	104.02	-
Acid	65.60	0.995816
Base	103.52	0.993310
Oxidation	98.48	0.999985
Heat	97.60	0.999987
Light	99.20	0.999971
UV	99.84	0.999978

Table 6c Drug product specificity- Amlodipine

Amlodipine		
Sample	% Assay	Peak purity
Unstressed	99.80	-
Acid	ND[a]	-
Base	85.60	0.962404
Oxidation	90.80	0.983756
Heat	99.80	0.992792
Light	76.80	0.987310
UV	96.00	0.988200

[a] No Data

Table 6d Specificity of sample and standard

	Olmesartan	Hydrochlorothiazide	Amlodipine
RT in Indian Injection	4.181	2.645	17.92
RT in Std	4.277	2.667	17.355
RT in Sample	4.245	2.656	17.269
Placebo		No Interference	
Bank		No Interference	

Forced degradation studies

Forced degradation studies were performed to establish stability indicating nature of the assay method. Tablet sample, Olmesartan Medoxamil, Hydrochlorothiazide, Amlodipine Besylate and Placebo (Excipients) to following stress conditions to undergo degradation. Table: 7.

Table 7 Forced degradation studies: Various stress conditions

Sample stress condition	Description of stress condition	Stress time
Acid degradation	5N HCl	2 h
Base degradation	5N NaOH	2 h
Oxidation degradation	20% v/v Hydrogen Peroxide	1 h
Photo degradation	Direct exposure to sunlight	12 h
Degradation by UV	Exposed to UV light	120 h
Thermal degradation	105 °C	24 h

Interference studies
The sample solvent and placebo solution were injected to demonstrate non-interference. Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Besylate peaks were identified by their retention times. Peak purity index for the analytes of interest were generated.

Olmesartan, Hydrochlorothiazide and Amlodipine peaks are free of interference in all the stress studies where as the analytical method is sensitive to the degradation (for Olmesartan, Hydrochlorothiazide and Amlodipine) and sensitive to base degradation (for Olmesartan alone). Henceforth the method is selective to Olmesartan, Hydrochlorothiazide and Amlodipine and their degradation production. Degradation product peaks did not interfere with the intact main peak, so this method is stability indicating method. Fig. 3A-3L

Linearity
Linearity of the analytical method was demonstrated by taking series of solution containing Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Besylate at concentration in the range 70-130% were analyzed to determine the linearity of the method. Each injection was injected in duplicate and the results were presented in Table: 8a, 8b and 8c. Fig.4-6. A plot was drawn between the concentrations versus peak response of Olmesartan, Hydrochlorothiazide, Amlodipine. The test method was demonstrated to be linear for all the three drugs in the concentration ranges listed above.

Table 8a Linearity- Olmesartan

Olmesartan		
% Concentration	Actual Conc ppm	Avg Area
70%	70.15	982328
80%	80.18	1116963
90%	90.20	1227873
100%	100.22	1353717
110%	110.24	1519465
120%	120.26	1644079
130%	130.29	1773033

Table 8b Linearity- Hydrochlorothiazide

Hydrochlorothiazide		
% Concentration	Actual Conc ppm	Avg Area
70 %	44.05	286741
80 %	50.34	302343
90 %	56.63	331513
100 %	62.93	365031
110 %	69.22	409350
120 %	75.51	440960
130 %	81.80	473530
Corr Coeff R[a]		0.999022

[a] *Correlation Coefficient*

Table 8c Linearity- Amlodipine

Amlodipine		
% Concentration	Actual Conc ppm	Avg Area
70 %	17.29	187052
80 %	19.76	223373
90 %	22.24	249396
100 %	24.74	274330
110 %	27.18	311207
120 %	29.65	334272
130 %	32.12	364191
Corr Coeff R[a]		0.998719

[a] *Correlation Coefficient*

Accuracy

Accuracy is the measure of exactness of an analytical method or closeness of agreement between the measured value and the value that is accepted either as true value, conventional or an accepted reference. It is generally measured as the percentage of analyte recovered through assay by spiking samples in a blind study. The accuracy of the method was determined by analyzing samples of placebo spiked with Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine at concentrations representing the levels listed above. The percent recovery results are summarized on Table 9a, 9b and 9c.

Table 9a Accuracy -Olmesartan

% Sample Concentration	Olmesartan		
	Amount added (mg/sample preparation)	Amount found (mg/sample preparation)	% Recovery
70 %	72.0	74.10	102.91
70 %	70.7	71.73	101.45
Mean % Recovery			102.18
% RSD[a]			1.01
100 %	99.6	102.14	102.55
100 %	97.5	97.92	100.43
Mean % Recovery			101.49
% RSD			1.48
130 %	132.9	136.71	102.87
130 %	131.9	133.66	101.33
Mean % Recovery			102.10
% RSD			1.07

[a] *Relative Standard Deviation*

Table 9b Accuracy- Hydrochlorothiazide

% Sample Concentration	Hydrochlorothiazide		
	Amount added (mg/sample preparation)	Amount found (mg/sample preparation)	% Recovery
70 %	45.6	46.70	102.41
70 %	43.8	45.00	102.75
Mean % Recovery			102.58
% RSD[a]			0.23
100%	62.9	64.47	102.50
100%	61.4	61.42	100.04
Mean % Recovery			101.27
% RSD			1.72
130 %	8.4	85.63	102.68
130 %	82.4	83.77	101.66
Mean % Recovery			102.17
% RSD			0.70

[a] *Relative Standard Deviation*

Table 9c Accuracy- Amlodipine

% Sample Concentration	Amlodipine		
	Amount added (mg/sample preparation)	Amount found (mg/sample preparation)	% Recovery
70 %	17.6	17.81	101.18
70 %	17.3	17.57	101.55
Mean % Recovery			101.37
% RSD[a]			0.26
100 %	25.5	25.55	100.18
100 %	25.3	25.71	99.48
Mean % Recovery			99.83
% RSD			0.50
130 %	32.8	32.68	99.63
130 %	32.6	32.31	99.12
Mean % Recovery			99.37
% RSD			0.36

[a] *Relative Standard Deviation*

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated by varying parameters such as percent organic solvent, pH, temperature, wavelength, mobile phase flow rate and determining the effect on the results of the method. Deliberate variations in the method parameters were made in order to demonstrate the robustness of the method. Results are presented in Table: 10a-10i, Fig. 2D.

Table 10a Robustness-Wavelength-Olmesartan

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	0.92	2231	99.30	0.60
237 nm	1.01	2263	99.90	-
239 nm	0.92	2232	99.70	0.20

Table 10b Robustness-Wavelength-Hydrochlorothiazide

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	1.16	2370	102.80	1.20
237 nm	1.17	2274	104.00	
239 nm	1.16	2357	102.80	1.20

Table 10c Robustness-Wavelength-Amlodipine

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	1.26	2327	100.00	0.20
237 nm	1.23	2872	99.80	-
239 nm	1.35	2465	100.40	0.60

Table 10d Robustness-Flow-Olmesartan

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	1.14	2228	100.60	0.70
237 nm	1.01	2263	99.90	-
239 nm	1.03	2383	101.00	1.10

Table 10e Robustness-Flow-Hydrochlorothiazide

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	1.21	2101	103.04	0.96
237 nm	1.17	2274	104.00	-
239 nm	1.30	2322	102.32	1.68

Table 10f Robustness-Flow-Amlodipine

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	1.16	2202	100.00	0.20
237 nm	1.23	2872	99.80	-
239 nm	1.34	2218	100.00	0.20

Table 10g Robustness-Column Temperature-Olmesartan

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	1.28	2188	99.25	0.65
237 nm	1.01	2263	99.90	-
239 nm	1.21	2772	99.85	0.05

Table 10h Robustness-Column Temperature-Hydrochlorothiazide

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	1.38	2487	102.64	1.36
237 nm	1.17	2274	104.00	-
239 nm	1.28	2457	102.64	1.36

Table 10i Robustness-Column Temperature-Amlodipine

Wave length	% Assay			
	Tailing factor	Theoretical plate count	% Assay	Absolute difference
235 nm	1.52	2181	100.20	0.40
237 nm	1.23	2872	99.80	-
239 nm	1.62	2760	99.40	0.40

Stability of standard and sample solution

The stability of the standard and sample were determined by making a series of standard and sample solutions over a period of about 16 h. Percentage Recovery analysis was performed on the peak areas of Olmesartan, Hydrochlorothiazide and Amlodipine respectively. Table: 11a-11b.

Table 11a Standard solution stability at room temperature

Interval	Olmesartan		Hydrochlorothiazide		Amlodipine	
	Area	% Recovery	Area	% Recovery	Area	% Recovery
Initial	1404113	-	369634	-	305303	-
4^{th} h	1403807	99.87	368748	99.76	307299	100.65
8^{th} h	1396571	99.48	364591	98.87	307380	100.02
12^{th} h	1401307	100.33	367548	100.81	306949	99.85
16^{th} h	1405247	100.28	368473	100.25	306347	99.80
Tailing Factor	0.96		1.07		1.09	
Theoritical plates	2132		2544		2455	
%RSD	0.38		0.28		0.43	

Table 11b Sample solution stability at room temperature

Interval	Olmesartan		Hydrochlorothiazide		Amlodipine	
	Area	% Recovery	Area	% Recovery	Area	% Recovery
Initial	1408351	100.30	369634	-	374271	-
4th h	1412948	100.63	368748	100.32	376797	100.67
8th h	1410059	100.43	364591	99.80	374054	99.27
12th h	1422979	101.35	367548	101.91	377060	100.80
16th h	1426291	101.58	368473	99.6	376233	99.97
Tailing Factor	1416125		1.07		375685	
Theoritical plates	8026		2544		1422	
%RSD	0.57		0.28		0.38	

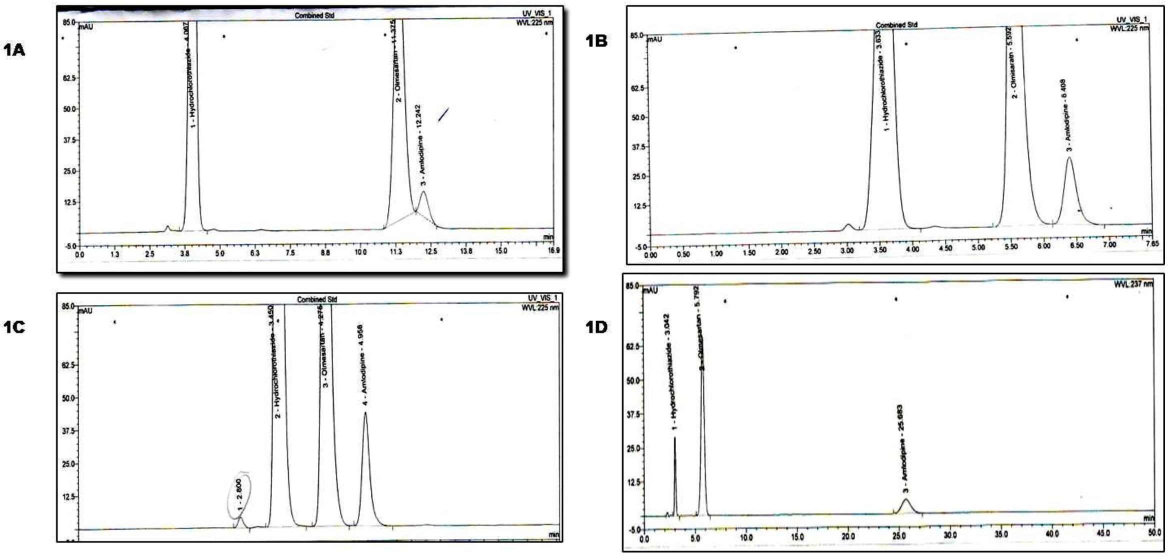

Fig.1 : [A] Buffer: Acetonitrile: Methanol composition at 70:10:30 [B] Buffer: Acetonitrile: Methanol composition at 60:20:20 [C] Buffer: Acetonitrile: Methanol composition at 50:40:10 [D] Buffer: Acetonitrile composition at 87:13.

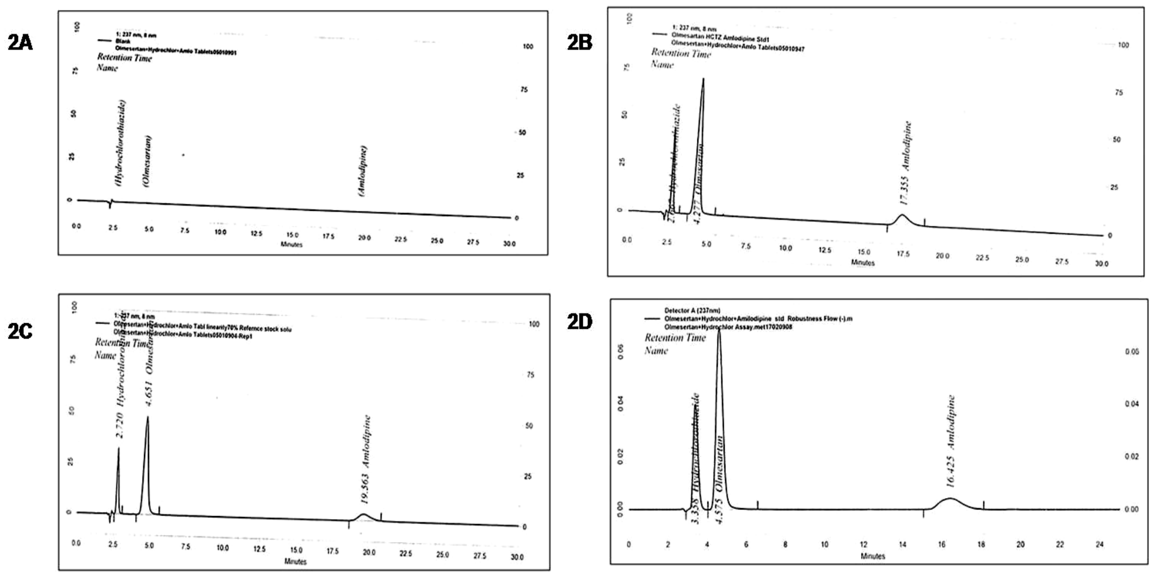

Fig.2: [A] Blank sample [B] Olmesartan, Hydrochlorothizide, Amlodipine (Standard) [C] Linearity for Olmesartan, Hydrochlorothizide, Amlodipine (Reference stock solution) [D] Olmesartan, Hydrochlorothizide, Amlodipine (Robustness flow (+) for sample).

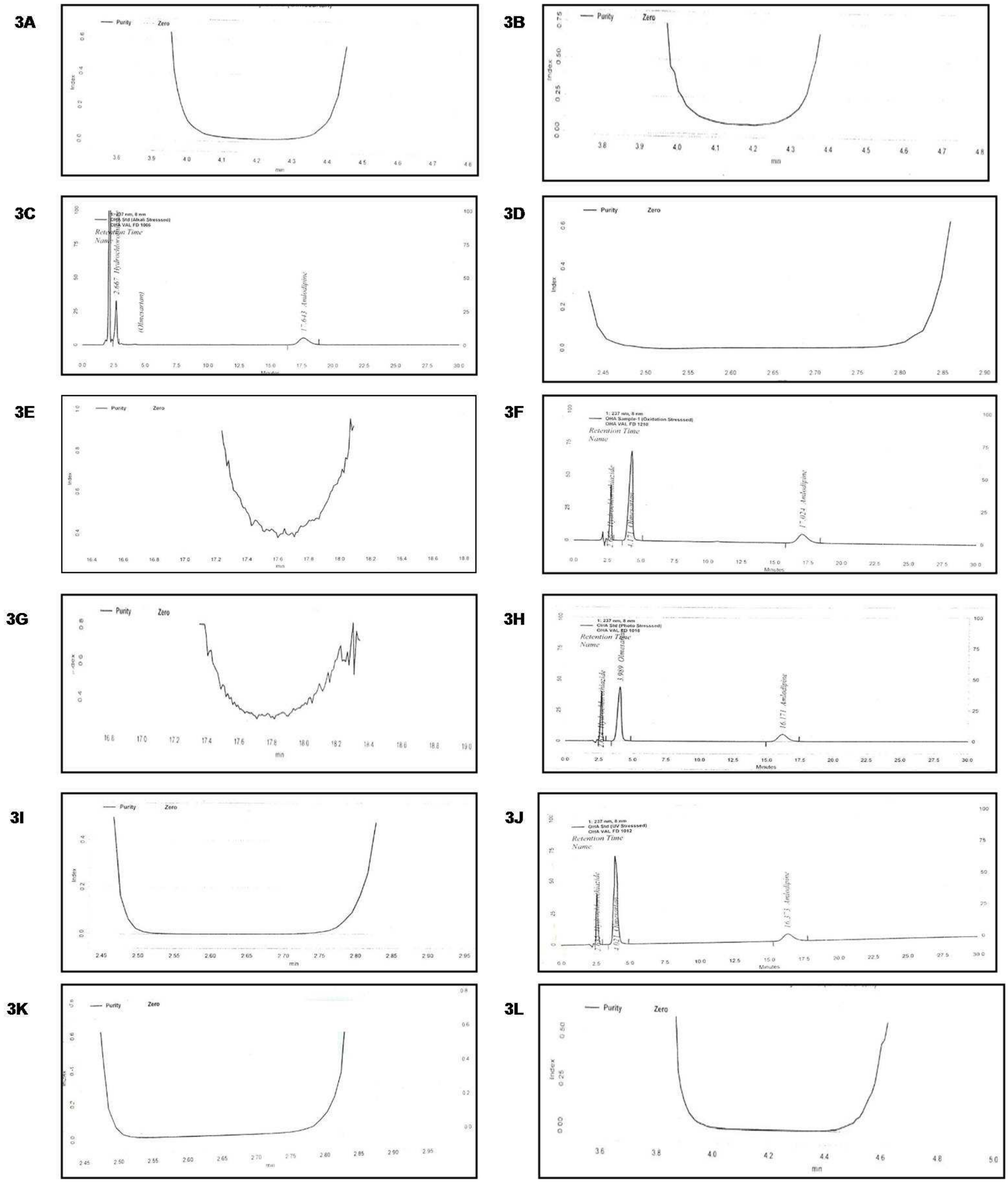

Fig.3: Chromatograms obtained during different stress conditions [A] Acid stressed for std: Purity curve of Olmesartan [B] Acid stressed for sample: Purity curve of Olmesartan [C] Alkali stressed for std [D] Alkali stressed for std : Purity curve of hydrochlorothiazide [E] Alkali stressed for std: Purity curve of Olmesartan [F] Oxidative stress for sample [G] Oxidative stress for sample: Purity curve of Olmesartan [H] Photo stressed for sample [I] Photo stressed for std: Purity curve of hydrochlorothiazide [J] UV stressed for std [K] UV stressed for sample : Purity curve of hydrochlorothiazide [L] Heat stressed for sample: Purity curve of Olmesartan.

This Reverse Phase HPLC is commonly used for the quantitative estimation of drugs from their formulation as well as from the biological fluids. Ease of performance specificity and analysis of sample are the important features of this technique. In the present work different proportion of organic solvents were tried for selection and optimization of mobile phase. Sincere attempt was made to increase the difference in retention time by increasing the proportion of Acetonitrile but decreasing the volume of buffer and methanol some extend resulted in the broadening of peaks. The mobile phase that found to be most suitable was Buffer: Acetonitrile (87:13 v/v) pH 7.0 with sodium hydroxide. This mobile phase composition gave good resolution and optimum retention time with appropriate tailing factor (< 2). The detection wave length was selected as 237.0 nm where the peaks height of the drugs was acceptable. Finally the validation of HPLC method was performed as per ICH guidelines.

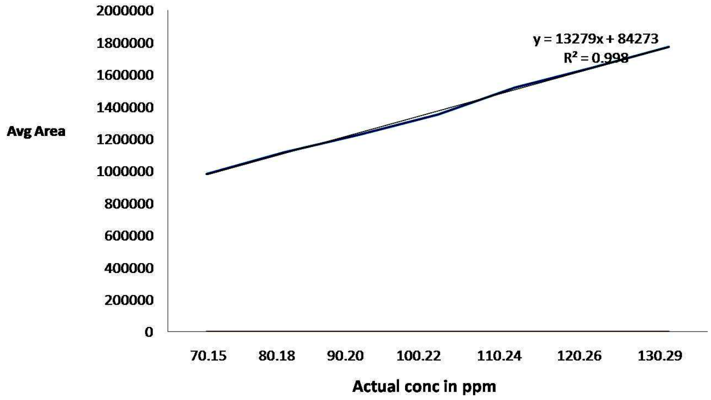

Fig.4: Linearity graph for Olmesartan Medoxomil.

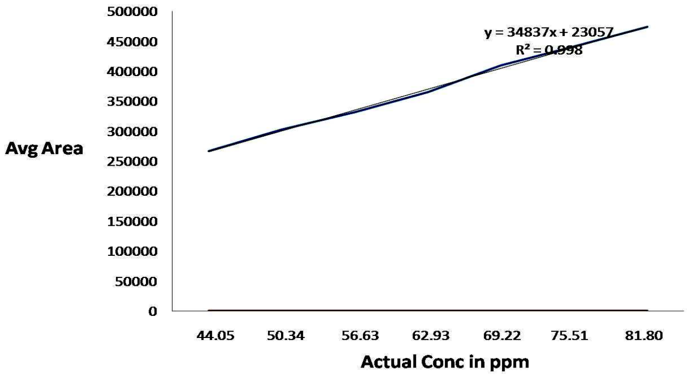

Fig.5: Linearity graph for Hydrochlorothiazide

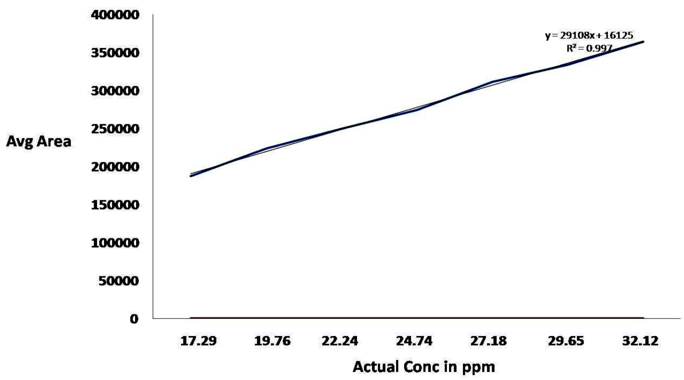

Fig.6: Linearity graph for Amlodipine

CONCLUSION

In conclusion successfully a robust HPLC analytical method was developed for the assay of Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine Besylate with respect to dosage form and method of analysis. This method is stability indicating method and therefore can be applied to the formulation for analysis of the sample for release and stability purpose. Similarly the interference of known impurities was studied during the development stage. The performance characteristics studied for the optimization of the method were Specificity, Precision, Accuracy, Robustness and Linearity.

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