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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF HYDROCHLOROTHIAZIDE, OLMESARTAN MEDOXOMIL AND THEIR RELATED SUBSTANCES IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

Objective: Development of RP-HPLC method for determination of Hydrochlorothiazide (HCTZ), Olmesartan medoxomil (OLM) and their related substances in combined tablet dosage form and validation of the developed method.

Methods: Gradient mobile phase system was used for estimation of drug contents and their related substances. Mobile phase A contained the mixture of Acetonitrile and 15 mM Phosphate buffer (pH adjusted to 3.4 with orthophosphoric acid) in the ratio of 20:80. Mobile phase B contained the same mixture in the ratio of 80:20. Chromatographic separation was carried out at the mobile phase flow rate of 0.8 mL/min using C_{18} Phenomenax inplace of Enable (250 × 4.6 mm) 5 µm column and detection was made at 254 nm.

Results: The linearity of developed method was tested in the range of 62.5-187.5 µg/mL for Hydrochlorothiazide, 100-300 µg/mL for Olmesartan medoxomil, 1-1.8 µg/mL for Hydrochlorothiazide. The % recovery was found to be 99.88-100.67 % (HCTZ), 99.14-99.91 % (OLM), 99.11-100.71% (HCTZ-IMP) and 98.13-100.83% (OLM-IMP). The assay of marketed formulation was found to be 99.78% (HCTZ) and 99.26% (OLM).

Conclusion: A simple, precise and accurate RP-HPLC method was developed for determination of Hydrochlorothiazide, Olmesartan medoxomil and their related substances.

Keywords: Hydrochlorothiazide (HCTZ), Olmesartan medoxomil (OLM), Hydrochlorothiazide Related Impurity (HCTZ-IMP), Olmesartan medoxomil Related Impurity (OLM-IMP), Reversed phase high performance liquid chromatography (RP-HPLC).

INTRODUCTION

Hydrochlorothiazide (HCTZ) is a diuretic antihypertensive drug. Chemically it is 6-chloro-3, 4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide clinically used in treatment of hypertension and management of edema. It inhibits the reabsorption of sodium in the distal convoluted tubule thereby decreasing the blood pressure.

Olmesartan medoxomil (OLM) is an antihypertensive drug. Chemically it is (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazole-5-carboxylate clinically used in the treatment of hypertension. Olmesartan is an angiotensin receptor blocker that selectively inhibits the binding of Angiotensin II to AT1. This results in decreased vascular resistance and blood pressure.

The literature review reveals various analytical methods for determination of Hydrochlorothiazide like UV [1,2], RP-HPLC [3,4], and HPTLC [5] and Olmesartan medoxomil UV [6,7], RP-HPLC [8,9] and HPTLC [10] individually as well as in combined dosage form with other drugs [11,12] but there was no method available for determination of these two drugs along with their related substances in combined tablet dosage form. There are various sources through which impurities (related substances & other impurities) may be generated in the drug product affecting its efficacy. It becomes necessary to have a method which can analyze such impurities in drug product.

Hydrochlorothiazide and Olmesartan medoxomil both have some related substance reported in pharmacopoeias [13,14]. They may be present in combined drug product in some amount or may get generated in the amount beyond the acceptance limit under worse conditions. So, the objective of present work was determination of Hydrochlorothiazide, Olmesartan medoxomil and their related substances in combined tablet dosage form by validated RP-HPLC method as per ICH Q2(R1) [15].

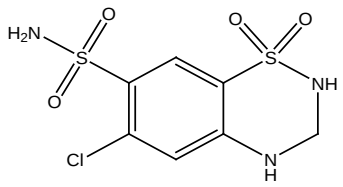

Fig. 1: Structure of HCTZ

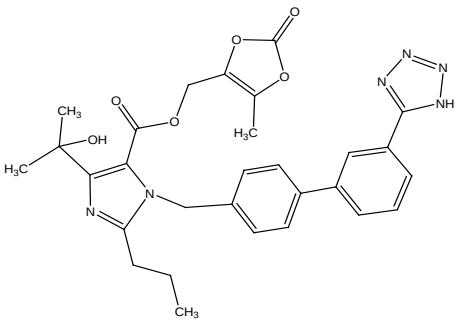

Fig. 2: Structure of OLM

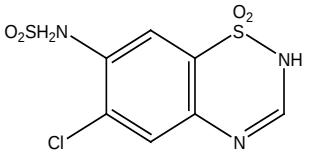

Fig. 3: Chlorothiazide (HCTZ-IMP)

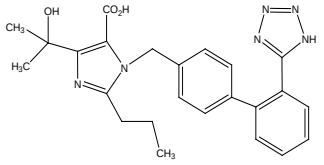

Fig. 4: Olmesartan acid impurity (OLM-IMP)

MATERIALS AND METHODS

Instrumentation

HPLC: Shimadzu LC-20AT system equipped with LC solution software, PDA detector, injection volume: 20µL, column: C_{18} Phenomenax inplace of Enable (250 × 4.6 mm) 5µm , Milli Q water purification system.

Materials

Standard gift samples of Hydrochlorothiazide, Olmesartan medoxomil and their related impurities were provided by Zydus Cadila Healthcare Ltd. Combined tablet dosage form OLMEZEST H-20 was purchased from local market. Acetonitrile (HPLC Grade) was procured from Loba Chemie Pvt Ltd, Mumbai. Potassium dihydrogen phosphate (HPLC Grade) was used for preparation of buffer solution.

Chromatographic conditions

The chromatographic column used was C_{18} Phenomenax inplace of Enable (250 × 4.6 mm) 5µm. The gradient method was employed with mobile phase A & B using 0.8 mL/min flow rate and 254 nm detection wavelength during entire gradient program. Injection volume was 20 µL.

Mobile phase A- Acetonitrile: 15 mM Phosphate buffer (pH 3.4) in the ratio of 20:80

Mobile phase B- Acetonitrile: 15 mM Phosphate buffer (pH 3.4) in the ratio of 80:20

Table 1: Gradient Program

Time (min)	A (% v/v)	B (% v/v)
0-26	87→13	13→87
26-27	13→87	87→13
27-30	87	13

Preparation of Solutions

Preparation of standard stock solutions

Stock solutions of 1250 µg/mL of HCTZ and 2000 µg/mL of OLM were prepared by dissolving 125 mg of HCTZ and 200 mg of OLM in 100 mL of acetonitrile in separate 100 mL volumetric flasks respectively.

HCTZ-IMP (4 mg) was accurately weighed and transferred to a 10 mL volumetric flask and then dissolved and diluted to 10 mL with acetonitrile. From above solution 1 mL was transferred to a 10 mL volumetric flask and diluted to 10 mL with acetonitrile to obtain stock solution of 40 µg/mL.

OLM-IMP (5 mg) was accurately weighed and transferred to a 10 mL volumetric flask and then dissolved and diluted to 10 mL with acetonitrile. From above solution 2 mL was transferred to a 10 mL volumetric flask and diluted to 10 mL with acetonitrile to obtain stock solution of 100 µg/mL.

Validation of developed method

The developed method was validated according to International Conference on Harmonization (ICH) Q2(R1) guideline.

System suitability

They are used to verify that resolution and reproducibility of chromatographic system are adequate for the analysis to be done. The parameters include Resolution (R), Tailing factor (T), Theoretical plates and Precision of replicate injection.

Linearity

Linearity of response was assessed by calibration curve in terms of slope, intercept and regression coefficient values. Five standard mixture solutions of HCTZ (62.5-187.5 µg/mL), OLM (100-300 µg/mL), HCTZ-IMP (1-1.8 µg/mL) and OLM-IMP (1-3 µg/mL) were analyzed for linear regression analysis.

Precision

Intraday and interday precision were performed over 3 levels of concentration at 3 different times in a day and on 3 different consecutive days respectively. Concentration levels of standard mixture of HCTZ, OLM, HCTZ-IMP and OLM-IMP used were 50%, 100% and 150% of the test concentration.

Specificity

Specificity for drug substances was assessed by taking the overlay of chromatograms of standard and sample. Specificity for impurities was assessed by taking the overlay of chromatogram of sample (i. e. drug product) and that of the mixture of impurity standards & drug substances.

Accuracy (n=3)

Accuracy was determined in terms of percentage recovery. Accuracy was determined by spiking 3 different known concentrations of standard to sample solution to obtain three levels of concentrations (80%, 100% and 120%).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were determined using following equations.

$LOD = 3.3\ \sigma/S$

$LOQ = 10\ \sigma/S$

Where, σ = Standard deviation of response

S = Slope of calibration curve

Robustness

The developed HPLC method was tested for robustness using factorial design (2^{3-1}) with four experiments. Three factors selected were Flow rate, Mobile phase ratio in line A and pH of buffer solution. The selection of factors was based on observation during method development. Each factor was studied at two levels. The levels of factors studied were selected according to error ranges which would be typically encountered in an analytical laboratory.

Table 2: Selected factors and levels

S. No.	Factor	Low level (-1)	High level (+1)
1.	Mobile phase ratio in line A	15:85	25:75
2.	pH of buffer solution	3.2	3.6
3.	Flow rate	0.7	0.9

Table 3: Factorial design 2^{3-1}

S. No.	Mobile phase in line A	pH of buffer solution	Flow rate
1.	15:85	3.2	0.7
2.	25:75	3.2	0.9
3.	15:85	3.6	0.9
4.	25:75	3.6	0.7

Analysis of marketed formulation

Twenty tablets were powdered and the powder quantity equivalent to 200 mg of OLM and 125 mg of HCTZ was accurately weighed and

transferred to a 100 mL volumetric flask. 50 mL of acetonitrile was added to dissolve the drug content and solution was filtered. The filtrate solution was diluted to 100 mL using acetonitrile. From above solution, 1 mL was transferred to a 10 mL volumetric flask and diluted to 10 mL with mixture of acetonitrile and water (50:50) to obtain final solution of 200 µg/mL (OLM) and 125 µg/mL (HCTZ). The amount of both the drugs was calculated from regression equation of calibration curve and percentage assay was calculated.

Forced degradation of marketed formulation

Sample solution of marketed formulation was prepared containing 2500 µg/mL OLM and 1562.5 µg/mL HCTZ. This solution was used as stock solution for degradation.

Acidic degradation

10 mL of above stock solution was transferred to a 100 mL volumetric flask and 10 mL of 0.1N HCl was added to it. Solution was kept for 60 min at room temperature and then it was neutralized. The final volume was made up to 100 mL using mixture of acetonitrile and water (50:50).

Alkaline degradation

10 mL of stock solution was transferred to a 100 mL volumetric flask and 10 mL of 0.1N NaOH was added to it. Solution was kept for 30 min at room temperature and then it was neutralized. The final volume was made up to 100 mL using mixture of acetonitrile and water (50:50).

Oxidative degradation

10 mL of stock solution was transferred to a 100 mL volumetric flask and 10 mL of 5% H_2O_2 was added to it. Solution was kept for 60 min at room temperature. The final volume was made up to 100 mL using mixture of acetonitrile and water (50:50).

The degraded samples were subjected to HPLC analysis using developed method and the percentage impurity of interest was calculated using following equation.

% Impurity = (Area of that impurity peak / Total Area of all peaks) * 100

RESULTS AND DISCUSSION

Optimization of chromatographic condition

The retention time was found to be 6.194 min (HCTZ), 8.389 min (HCTZ-IMP), 15.226 min (OLM) and 19.309 min (OLM-IMP).

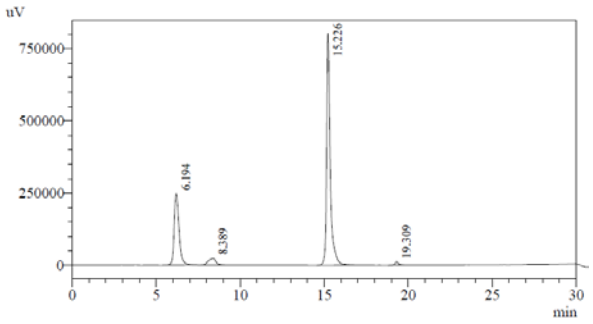

Fig. 5: Chromatogram of Mixture of HCTZ, HCTZ-IMP, OLM & OLM-IMP (125, 1.4, 200 & 2 µg/mL in ACN:Water-50:50 respectively)

System Suitability Test

Results of theoretical plate, tailing factor, resolution and precision of injection repeatability have been shown below (Table 4).

Table 4: Data of System Suitability Test (n=6)

Parameter	HCTZ	OLM	HCTZ-IMP	OLM-IMP
Retention Time (min) ±SD	6.194±0.021	15.16±0.115	8.380±0.014	19.279±0.053
Tailing Factor ±SD	1.343±0.030	1.481±0.065	0.929±0.043	1.346±0.014
Theoretical Plate ±SD	2363.281 ± 214.089	29127.175 ± 1128.663	1797.433 ± 91.445	51571.147 ± 1031.876
Resolution	-	11.908	3.373	11.717
%RSD (Injection repeatability)	0.95	1.72	0.64	0.54

Linearity

The data of linearity of HCTZ, OLM, HCTZ-IMP and OLM-IMP has been shown below (Table 5). The results are within the acceptance criteria.

Precision

The % RSD for Intraday and Interday precision were found to be less than 2 for HCTZ, OLM, HCTZ-IMP and OLM-IMP which indicates that the developed method is precise.

The results of precision study have been shown below (Intraday: Table 7 & Table 8) (Interday: Table 9 & Table 10).

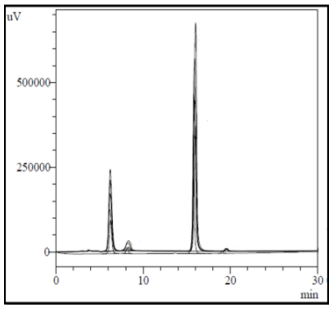

Fig. 6: Overlay Chromatogram for Linearity of HCTZ, OLM, HCTZ-IMP, OLM-IMP.

Specificity

Overlay of chromatograms for specificity has been shown below (fig. 7 and fig. 8).

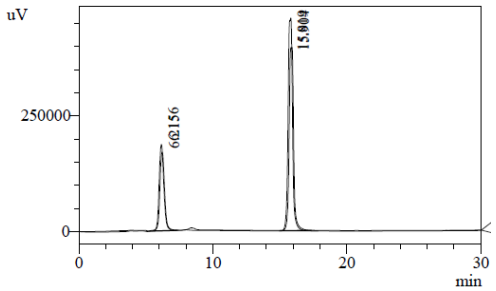

Fig. 7: Overlay Chromatogram of Standard & Sample for Specificity of HCTZ & OLM.

Accuracy

Accuracy study was performed at three levels for HCTZ, OLM, HCTZ-IMP and OLM-IMP (Table 11 and 12). The values of % recovery were found in the acceptance limit of 98-102 % with low % RSD, which justifies that, the method is accurate and free from the interference of excipients used in formulation and is applicable for analysis of marketed formulation.

Table 5: Linear regression data of HCTZ, OLM, HCTZ-IMP, OLM-IMP

Parameter	HCTZ	OLM	HCTZ-IMP	OLM-IMP
Regression equation	y = 30,571.4944x + 291,450.4	y = 47,953.4380x - 57,777.4	y = 188,952.50x - 73,059.9	y = 35,586.80x + 104,769.8
Regression coefficient mean ±SD	0.997±0.00037	0.998±0.00032	0.999±0.00030	0.997±0.00089
Mean of intercept ±SD	291,450.4 ± 12269.537	57,777.4 ± 3303.837	73,059.9 ± 2436.1	104,769.8 ± 1141.972
95% Confidence interval for intercept	277565.705 to 305335.095	54038.647 to 61516.153	70303.113 to 75816.687	103477.499 to 106062.101
Slope ±SD	30,571.4944 ± 196.99	47,953.4380 ± 489.83	188,952.50 ± 1563.52	35,586.80 ± 692.49
95% Confidence interval for Slope	30185.3944 to 30957.5944	46993.372 to 48913.504	185888.001 to 192016.999	34803.151 to 36370.449

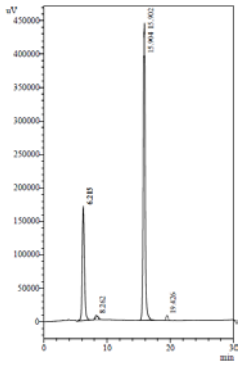

Fig. 8: Overlay Chromatogram of Standard spiked with impurities and Sample for Specificity of HCTZ-IMP & OLM-IMP

Table 6: LOD and LOQ data

	HCTZ	OLM	HCTZ-IMP	OLM-IMP
LOD (µg/mL)	1.32	5.08	0.04	0.10
LOQ (µg/mL)	4.01	15.39	0.13	0.32

Robustness

The effect of 3 factors on the response was analyzed using design expert software and p-value was obtained for each factor. The p-value for factors Mobile phase ratio in line A and pH of buffer solution were found to be greater than 0.05 which indicates that they are non significant factors within the study range of factors for robustness. The p-value of factor Flow rate was found to be less than 0.05 for HCTZ and OLM which indicates that flow rate is a significant factor affecting the response of HCTZ and OLM.

Table 7: Intraday Precision Data of HCTZ & OLM (n=3)

HCTZ			OLM		
Conc. (µg/mL)	**Mean Area ± SD**	**%RSD**	**Conc. (µg/mL)**	**Mean Area ± SD**	**%RSD**
62.5	2098427 ± 4553.48	0.22	100	4585538 ± 1659.25	0.04
125	4144189 ± 9575.98	0.23	200	9648941 ± 83119.86	0.86
187.5	5967782 ± 24816.77	0.42	300	14137073 ± 65355.89	0.46

Table 8: Intraday Precision Data of HCTZ-IMP & OLM-IMP (n=3)

HCTZ-IMP			OLM-IMP		
Conc. (µg/mL)	**Mean Area ± SD**	**%RSD**	**Conc. (µg/mL)**	**Mean Area ± SD**	**%RSD**
1	114895 ± 1537.68	1.34	1	138950 ± 425.61	0.31
1.4	192754 ± 614.51	0.32	2	175622 ± 88.15	0.05
1.8	265152 ± 170.28	0.06	3	211829 ± 1407.87	0.66

Table 9: Interday Precision Data of HCTZ & OLM (n=3)

HCTZ			OLM		
Conc. (µg/mL)	**Mean Area ± SD**	**%RSD**	**Conc. (µg/mL)**	**Mean Area ± SD**	**%RSD**
62.5	1930766 ± 10293.61	0.53	100	4543694 ± 56438.02	1.24
125	4090065 ± 39388.14	0.96	200	10149030 ± 119970.5	1.18
187.5	6222916 ± 654.82	0.01	300	15401259 ± 39407.02	0.26

Table 10: Interday Precision Data of HCTZ-IMP & OLM-IMP (n=3)

HCTZ-IMP			OLM-IMP		
Conc. (µg/mL)	**Mean Area ± SD**	**%RSD**	**Conc. (µg/mL)**	**Mean Area ± SD**	**%RSD**
1	113193 ± 2015.28	1.78	1	121755 ± 1495.97	1.22
1.4	215568 ± 1808.22	0.84	2	180156 ± 1315.37	0.73
1.8	281837 ± 1262.29	0.46	3	220241 ± 2198.62	0.99

Analysis of marketed formulation

Marketed formulation OLMEZEST H-20 containing 20 mg of OLM and 12.5 mg of HCTZ was used for assay purpose. Tablet powder was dissolved, filtered and diluted to make the final concentration of 200 µg/mL (OLM) and 125 µg/mL (HCTZ). Along with the peaks of HCTZ and OLM, a small peak at 8.386 min was obtained which is a peak of HCTZ Related substance (i. e. HCTZ-IMP) present in marketed formulation.

Forced degradation of marketed formulation:

The tablet sample was subjected to acidic, basic and oxidative degradation to generate the impurities in sample. The impurities of

interest were quantified using regression equation obtained from calibration curves of impurity standards. In all three conditions for degradation of marketed formulation, HCTZ-IMP was generated in more amounts and OLM-IMP was generated in very less amount because the chromatogram of marketed formulation without degradation shows that the HCTZ Related Impurity was already present within the acceptance limit and after degradation its amount got increased.

Table 11: Accuracy Data of HCTZ & OLM

	Sample (µg/mL)	Std added (µg/mL)	Total (µg/mL)	Mean Area ± SD	% RSD	Total found (µg/mL)	% Reco-very
	75	60	135	4446142±54748.6	1.23	135.90	100.67
HCTZ	75	75	150	4871521±57179.3	1.17	149.82	99.88
	75	90	165	5354073±43326.3	0.81	165.60	100.36
	120	96	216	10211399±139896.2	1.37	214.15	99.14
OLM	120	120	240	11370489±89519.63	0.79	238.32	99.30
	120	144	264	12591083±10306.66	0.08	263.77	99.91

Table 12: Accuracy Data of HCTZ-IMP & OLM-IMP

	(HCTZ + OLM) (µg/mL)	Impurity Std spiked (µg/mL)	Mean Area±SD	% RSD	Spiked Std found (µg/mL)	% Recovery
HCTZ IMP	125 + 200	1.12	137886±822.50	0.59	1.11	99.11
	125 + 200	1.4	192577±622.73	0.32	1.41	100.71
	125 + 200	1.68	243042±1200.65	0.49	1.67	99.40
OLM IMP	125 +200	1.6	160769±1087.36	0.67	1.57	98.13
	125 + 200	2	175133±434.17	0.25	1.97	98.50
	125 + 200	2.4	191004±763.04	0.39	2.42	100.83

Table 13: p-values of factors for robustness

	p-value of factors		
	Mobile Phase Ratio in line A	pH of buffer solution	Flow rate
HCTZ	0.9166	0.8075	0.0223
OLM	0.9700	0.8175	0.0173
HCTZ-IMP	0.7276	0.2360	0.4151
OLM-IMP	0.6794	0.7586	0.0841

p-value < 0.05: Factor has significant effect on response, p-value > 0.05: Factor has no significant effect on response

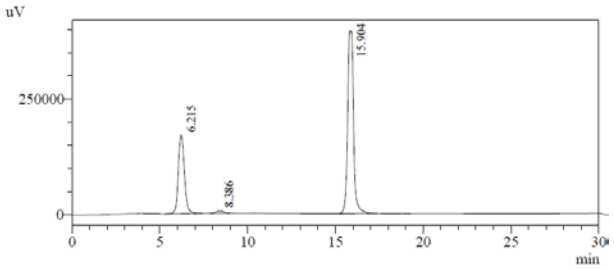

Fig. 9: Chromatogram of marketed formulation

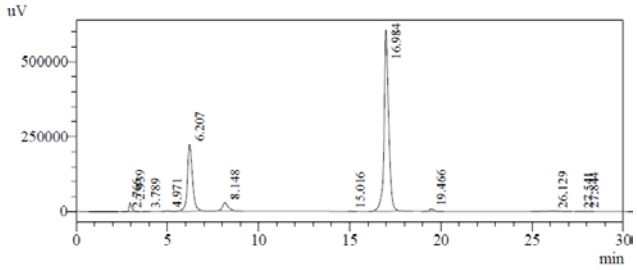

Fig. 11: Alkaline degradation of tablet sample

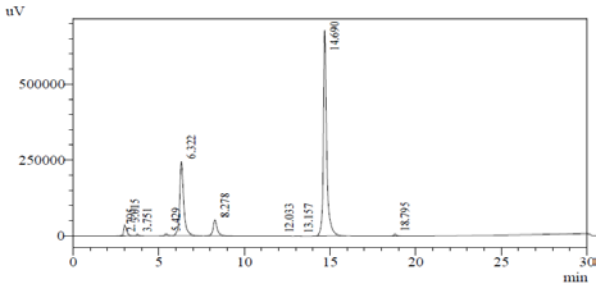

Fig. 10: Acidic degradation of tablet sample

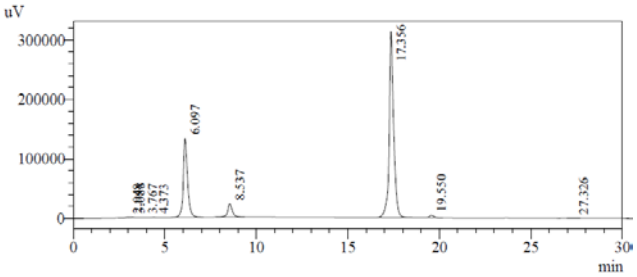

Fig. 12: Oxidative degradation of tablet sample

Table 14: Data of Marketed formulation Analysis

	Mean Area±SD	% RSD	% Assay
HCTZ (125 µg/mL)	4104467±5848.43	0.14	99.78 %
OLM (200 µg/mL)	9461639±88939.41	0.94	99.26 %
HCTZ-IMP	139486±727.99	0.52	1.01 %

Table 15: Acidic degradation data

Impurity	Retention Time (min)	Peak Area	Conc. found (µg/mL)	% Impurity
HCTZ-IMP	8.278	850913	4.89	5.79 %
OLM-IMP	18.795	110533	0.16	0.75 %

Table 16: Alkaline degradation data

Impurity	Retention Time (min)	Peak Area	Conc. found (µg/mL)	% Impurity
HCTZ-IMP	8.148	664499	3.90	4.10 %
OLM-IMP	19.466	119840	0.42	0.74 %

Table 17: Oxidative degradation data

Impurity	Retention Time (min)	Peak Area	Conc. found (µg/mL)	%Impurity
HCTZ-IMP	8.537	396041	2.48	4.94 %
OLM-IMP	19.550	117986	0.37	1.47 %

CONCLUSION

The developed method was validated as per ICH Q2(R1) guideline and was found to be within the prescribed limit. It concludes that the developed method is simple, accurate, sensitive and precise and suitable for routine quality control analysis of Hydrochlorothiazide, Olmesartan medoxomil and their related substances in combined tablet dosage form.

CONFLICT OF INTERESTS

Declared None

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