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Research

Related Impurities HPLC Method Development & Validation for drug combinations: Olmesartan Medoxomil, Chlorthalidone & Cilnidipine

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ABSTRACT

The LC-MS compatible, stability-indicating, specific, linear, accurate, sensitive with less run-time RP-HPLC related impurities method has been developed for Olmesartan Medoxomil, Chlorthalidone, and Cilnidipine drug combinations. And the method has been validated according to ICH and US-FDA guidelines. The chromatographic separation was performed by using Hypersil-BDS Thermo-Scientific, C/18 (12.5 cm, 4.6mm, 5-micron particle size) column. Mobile phase-I was prepared by mixing 3.85 gm Ammonium acetate in HPLC water and adjust pH-5.0 by using diluted acetic acid. Acetonitrile was taken as Mobile phase-B. Initial mobile phase ratio (55:45,v/v) was adjusted for Mobile phase-A: Mobile phase-B followed by gradient program. Other chromatographic conditions such as column temperature 25 degrees, flow rate 1.0 mL/minutes with the detection wavelength at 260 nm. The retention time for Chlorthalidone Impurity A, Olmesartan, Olmesartan Medoxomil Impurity A, were found about 2.7, 3.3, and 7.2 minutes respectively, with a total run time of 18.0 minutes. The linearity calibration plot was performed and found linear relationship over the concentration range of 1.25(LOQ)–18.75 µg/ml, 3.6(LOQ)–60.0 µg/ml, 3.6(LOQ)–60.0 µg/ml respectively for Chlorthalidone Impurity-A, Olmesartan and Olmesartan Medoxomil Impurity A respectively. The LOD and LOQ were found 0.4 ppm (µg/ml) & 1.2 ppm (µg/ml), 1.2 ppm (µg/ml) & 3.5 ppm (µg/ml), 1.1 ppm (µg/ml) & 3.3 ppm (µg/ml) for Chlorthalidone Impurity A, Olmesartan and Olmesartan Medoxomil Impurity A respectively. The accuracy was determined by recovery studies and was found between 90.0-110.0%. The developed analytical method has been validated for lod-loq, specificity, linearity, accuracy, precision, robustness, and ruggedness, which were well within the acceptance limit as per ICH guidelines. All the degradation products generated by stress conditions were found to be well separated from one another (all drug components and impurities). The developed method with shorter runtime was successfully implemented for routine quality control and stability analysis to check the quality of olmesartan medoxomil, chlorthalidone, and cilnidipine drug combinations.

Content:

INTRODUCTION

Olmesartan Medoxomil (OLM) (Fig. 1). Olmesartan Medoxomil is a synthetic imidazole derivative pro-drug with an antihypertensive property. OLM prevents angiotensinII induced vasoconstriction and decreases aldosterone production, thereby preventing aldosterone-stimulated sodium retention and potassium excretion.

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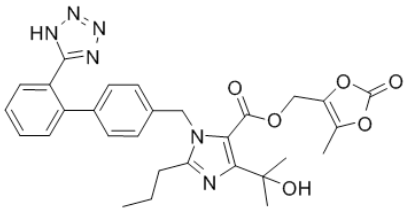

Fig. 1: Chemical structure of Olmesartan Medoxomil

Chlorthalidone (CHLR) Fig. 2]. Chlorthalidone is a diuretic medication used to treat high blood pressure, swelling including that due to heart failure, liver failure and nephrotic syndrome, diabetes insipidus, and renal tubular acidosis. Cilnidipine (CIL) (Fig. 3). Cilnidipine is a calcium channel blocker. Cilnidipine decreases blood pressure and is used to treat hypertension and its comorbidities. Olmesartan Medoxomil, Chlorthalidone and Cilnidipine combinations are used to treat hypertension when a single medication is not effective. It also helps to reduce chances of future heart attack and stroke. The most important related compounds for Olmesartan Medoxomil are Olmesartan and Olmesartan Medoxomil impurity-A, for Chlorthalidone is Chlorthalidone impurity-A. A literature survey discloses that few stability-indicating HPLC methods,[1-18] HPTLC,[19-20] Spectrophotometric methods[21-22] methods have been reported for the estimation of Olmesartan medoxomil and or Chlorthalidone and or Cilnidipine along with drug combinations in pharmaceutical preparations. To best to our knowledge, no reports were found for stability-indicating LC-MS compatible Related Impurities method for Olmesartan Medoxomil, Chlorthalidone & Cilnidipine drug combinations. In the present work, we are concentrated on to develop and validate a stability-indicating, LC-MS compatible method(with less runtime) along with optimum chromatographic conditions for the determination of related impurities (Olmesartan, Olmesartan Medoxomil Impurity-A, Chlorthalidone Impurity-A, and un-known impurities) for Olmesartan Medoxomil, Chlorthalidone & Cilnidipine drug combinations that may be present during stability study. The developed LC-MS compatible method was validated as per ICH guidelines[23-24] and can be applied lucratively to quality control purposes.

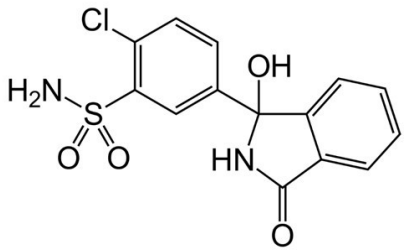

Fig. 2: Chemical structure of Chlorthalidone

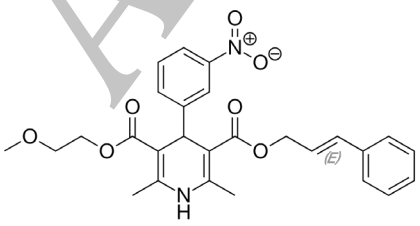

Fig. 3: Chemical structure of Cilnidipine

Materials and Methods

Materials

A pharmaceutical-grade gift sample of Olmesartan Medoxomil (established purity 99.2%), Chlorthalidone (purity 98.8%), Cilnidipine (purity 99.5%) were acquired from Amoli Organics PVT LTD. Olkem Trio 40 tablets containing Olmesartan Medoxomil 40 mg, Chlorthalidone 12.5 mg and Cilnidipine 10 mg were procured from the domestic market. Water HPLC grade, Acetonitrile HPLC grade, and Methanol HPLC grade were purchased from Merck. HPLC grade of Glacial acetic acid and Ammonium acetate were procured from Merck.

Methods

Instrumentation

LC-20AT (Shimadzu) system was used for HPLC method development & validation by using Hypersil BDS, C/18 (12.5 cm × 0.46 cm) 5-micron column, as well as UV-Visible detector, analyzed at 260 nm. Spinchrom Software was used for evaluation and data processing.

Chromatographic Conditions

A mobile phase-A was prepared by dissolving 3.85-gram ammonium acetate into 1-liter water. Adjust pH 5.0 with diluted Acetic acid and filter through a 0.22-micron membrane filter, sonicated for 10 minutes for degassing. Mobile phase-A kept for a line, and Acetonitrile kept for B line with the initial ratio of Mobile phase-A 55% and Acetonitrile 45%, prepared gradient program in the software (Table-1).

The analysis was carried out on LC-20AT (Shimadzu) system. The analytes was separated on an analytical column Hypersil BDS C18 (12.5 cm × 0.46 cm) 5 μm column at 260 nm wavelength. The column temperature was kept at 25°C. The volume of injection was 20 µL, and the flow was sustained at 1.0 mL/minutes. The runtime was 15 minutes and after that, 3 minutes saturation time with initial mobile phase ratio.

Diluent: Ammonium acetate buffer pH-5.0: Acetonitrile (55:45)

Table 1: Gradient program

Time	*Mobile phase-A (%)*	*Acetonitrile-B (%)*
0-2	55	45
2-4	65	35
4-15	10	90
15-18	55	45

Preparation of Standard Solution

- Chlorthalidone Impurity-A {CHLR impurity-A} stock solution (125µg/mL): Weigh accurately about 12.5mg of CHLR impurity-A and transfer to a 100mL volumetric flask. Add 60 ml methanol, sonicate till dissolve, and make up the volume up to the mark with methanol.
- Olmesartan {OL} stock solution (400µg/mL): Weigh accurately about 40mg of OL and transfer to a 100mL volumetric flask. Add around 60 mL Methanol, sonicate to dissolve, and make up the volume up to the mark with Methanol.
- Olmesartan Medoxomil Impurity-A {OLM impurity-A} stock solution (400µg/mL): Weigh accurately about 40mg of OLM impurity-A and transfer into a 100mL volumetric flask. Add about 60 mL methanol, sonicate to dissolve, and makeup to the mark with methanol.
- Preparation of Impurity solution of mixtures of CHLR impurity-A(12.5µg/mL),OL(40µg/mL) and OLM impurity-A (40µg/mL): Take 1 mL CHLR impurity-A stock solution, 1mL OL stock solution and 1mL OLM impurity-A stock solution, transfer to 10 mL volumetric flask, and make up the volume up to the mark with diluent and mix well.
- Chlorthalidone {CHLR} standard stock solution (125µg/mL): Weigh accurately about 12.5mg of CHLR and transfer to a 100mL volumetric flask. Add 60 ml methanol, sonicate till dissolve, and makeup volume up to the mark with methanol.
- Olmesartan Medoxomil {OLM} standard stock solution (400µg/mL): Weigh accurately about 40mg of OLM and transfer in 100mL volumetric flask. Add about 60 mL Methanol, sonicate to dissolve, and makeup volume up to the mark with methanol.
- Cilnidipine {CIL} standard stock solution (100µg/mL): Weigh accurately about 10mg of CIL and transfer in a 100mL volumetric flask. Add about 60 mL methanol, sonicate to dissolve, and makeup volume up to the mark with methanol.
- Preparation of solution mixtures of CHLR (12.5µg/mL), OLM (40µg/mL) and CIL(10µg/mL): Take 1 mL CHLR stock solution, 1mL OLM stock solution, and 1mL CIL stock solution, transfer to 10 mL volumetric flask and makeup to the mark with diluent, mix well.

Sample Solution Preparation

Weigh, powdered 20 tablets and the average weight was determined. Tablets were crushed by mortar-pastel and mixed well. Accurately weighed tablet powder 40 mg equivalent of OLM into a 10mL volumetric flask. Add 8mL diluent, shake for 15 minutes and sonicate the solution for 10 minutes. Makeup the volume with diluent and mix well to obtain Olmesartan Medoxomil (4000 μg/mL), Chlorthalidone (1250 μg/mL), and Cilnidipine (1000 μg/mL). Filter this solution with a 0.45µm membrane filter.

Method Validation

This method was validated as per USP and ICH guidelines. All validation parameters, eg. Specificity, sensitivity (LOQ and LOD) linearity-range, precision, accuracy, and robustness are included in the study.

Specificity

Specificity is one of the substantial features of HPLC, and it denotes the ability of the analytical method to separate analytes from one another in the complex mixture. Specificity of the method was performed by injecting 20 μL solutions of impurity, sample, and blank solutions individually.

Linearity.

To assess the linearity-range of the method, different solutions were prepared by diluting stock solutions with the diluent in different concentrations of Olmesartan impurity, Olmesartan Medoxomil Impurity-A and Chlorthalidone Impurity-A to achieve LOQ, 50%, 75%, 100%, 125% and 150% with respect to sample concentration respectively. One injection from each concentration was analyzed by using the same conditions. Linearity was plotted by using a linear regression method to evaluate r^2.

Sensitivity

LOD *(*Limit of detection) & LOQ (limit of quantitation) of Olmesartan impurity, Olmesartan Medoxomil Impurity-A, and Chlorthalidone Impurity-A were performed by preparing different solutions of Olmesartan impurity, Olmesartan Medoxomil Impurity-A, and Chlorthalidone Impurity-A and determine the S/N ratio. LOD is the lowest detection concentration with S/N ratio of approximately 3:1, while LOQ is the lowest quantification concentration with S/N ratio of approximately 10:1 along with %RSD (*n*= 5) of not more than 15%.

Accuracy

Accuracy of the related impurities method was determined by recovery studies at four levels of concentration (LOQ, 80.0%, 100.0%, and 120.0%) for Olmesartan impurity, Olmesartan Medoxomil Impurity-A, and Chlorthalidone Impurity-A and triplicate samples for individual concentration were injected. The recovery (%) for added Olmesartan impurity, Olmesartan Medoxomil Impurity-A and Chlorthalidone Impurity-A and RSD were measured for individual replicate samples.

Precision

The system precision & repeatability (method precision) for proposed methods were performed by multiple measurements of standard & sample solution, individually. A system precision was performed by five injections of the standard on the same day. Method precision was assessed by five injections of the sample on the same day. The RSD of the obtained results was calculated to evaluate repeatability results.

Robustness

Robustness study was performed for deliberate and minor modifications in the instrumental parameters, for example:

- Change in Flow: ± 0.2 mL/minutes
- Variation in organic composition (± 2.0)
- pH of Buffer: ± 0.2

The alteration was made to evaluate its impact on the method. The %RSD and difference in percentage was verified against original data for each of the modified parameters.

Results and Discussion

The study was aimed to develop a sensitive, accurate, precise, stability-indicating LC-MS compatible Related Impurities method for Olmesartan Medoxomil, Chlorthalidone & Cilnidipine drug combinations. A Hypersil BDS, C/18 (12.5 cm × 0.46 cm) 5-micron column was selected as the stationary phase for the separation and determination of related impurities method for Olmesartan Medoxomil, Chlorthalidone & Cilnidipine drug combinations. For the optimization of the mobile phase, sequential trials were performed by changing the ratio of methanol with water, acetonitrile with water, and buffer (ammonium acetate) with acetonitrile by isocratic as well as gradient program and monitored at different ratios. Method optimization results are summarized in Table 2.

Based on the above trails, the mobile phase containing ammonium acetate (pH-5.00) for A-line and acetonitrile for B line with initial ratio 55: 45 v/v and gradient program was finalized as per below table(Table-3).

Method was optimized with flow rate of 1.0 mL/minutes, wavelength 260.0 nm, 20 μL volume of injection and 25.0°C column temperature as the best chromatographic conditions for the complete study where Olmesartan Medoxomil, Chlorthalidone, Cilnidipine, Olmesartan impurity, Olmesartan Medoxomil Impurity A and Chlorthalidone Impurity A were eluted forming symmetrical peak shape and good resolution (Fig. 4).

Method Validation

Specificity

Specificity was assessed by comparing the chromatograms of blank, standard solution (Olmesartan Medoxomil, Chlorthalidone and Cilnidipine), Impurity standard

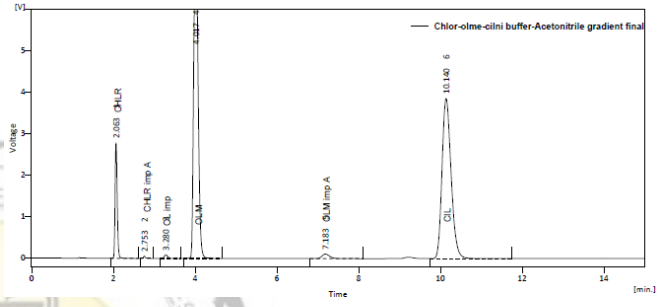

Fig. 4: Sample (spiked) chromatogram:

Table 3: Final Gradient program

Time	*Mobile phase-A (%)*	*Acetonitrile-B (%)*
0-2	55	45
2-4	65	35
4-15	10	90
15-18	55	45

Table 2: Method Development Summary:

Sr. No	*Mobile Phase*	*Remarks*
1	Water: Methanol (50:50)	Peak shape of CHLR and OLM observed are not good.
2	Water: Methanol (30:70)	Retention time reduced, but peak shape is not good for OLM.
3	Water: Methanol (10:90)	Peak for CHLR and OLM peak are merged.
4	Water: Acetonitrile (10:90)	Peak shapes were sharp for CHLR, OLM, and CIL, but no impurities are separated.
5	Buffer: Acetonitrile (50:50)	Peak of OL and OLM Imp-A are separated, but peak of CIL no observed.
6	Buffer: Acetonitrile (30:70)	Peak of OL and OLM Imp-A are separated, but peak of CIL not observed.
7	Buffer: Acetonitrile (20:80)	Peak of OLM and CHLR-A are merged.
8	Gradient-1 1) Buffer (pH-5.0): Acetonitrile (55:45) up to 2 minutes. 2) linear gradient to achieve Buffer: Acetonitrile (65:35) at 4 minutes. 3) linear gradient to achieve Buffer: Acetonitrile (10:90) at 15 minutes.	All analyte peak shapes are good and well separated from one another.
9	Gradient-2 1) Buffer (pH-5.0): Acetonitrile (55:45) up to 4 minutes. 2) linear gradient to achieve Buffer: Acetonitrile (20:80) at 14 minutes.	Trials are taken to reduce run time but CHLR Imp-A and OLM are very close to each other.
10	Gradient-3 1) Buffer (pH-5.0): Acetonitrile (50:50) up to 4 minutes. 2) linear gradient to achieve Buffer: Acetonitrile (20:80) at 15 minutes.	Trials are taken to reduce run time, but CHLR Imp-A and OLM are very close to each other.

(Olmesartan and Olmesartan Medoxomil Impurity-A, Chlorthalidone Impurity-A), as such sample and sample spiked with Olmesartan, Olmesartan Medoxomil Impurity-A & Chlorthalidone Impurity-A impurities solution. For the same purpose, 20 μL injection of diluent, standard, impurity standard solution, as such sample solution and sample spiked with Olmesartan, Olmesartan Medoxomil Impurity-A and Chlorthalidone Impurity-A impurities sample solution were injected into the HPLC system individually, and the chromatogram are shown in Figures 5–9. It can be observed that there no co-eluting peaks at the retention time of Olmesartan Medoxomil, Chlorthalidone, Cilnidipine, Olmesartan, Olmesartan Medoxomil Impurity-A and Chlorthalidone Impurity-A. All analyte peaks were pure and hence proved the specificity of the method.

Linearity and Range

Analytical method linearity is demonstrated as the ability of the method to get test results that are directly proportional to the concentration of analyte within a defined range. The peak area achieved from HPLC was plotted against respective concentrations to get the calibration graph. The results of linearity parameter Fig. 10-12 gave linear relationship over the concentration Range for CHLR impurity A, OL, and OLM Impurity A were assessed with concentration range from LoQ (1.25µg/mL)-18.75µg/mL, LOQ (3.6µg/mL)-60µg/mL and LOQ (3.6µg/ml)-60µg/ml respectively. Based on regression calculation, a linear equation was obtained: $y = mx + c$, and $r2$ was found greater than 0.990, representative a linear relationship for the concentration of analytes and peak area (Figures 10–12).

Limit of Detection and Limit of Quantification (LoD and LoQ)

The LOD is the lowest analyte level in a sample that could be detected, but not certainly quantitated and LoQ is the lowest analyte level in a sample can be precisely quantified.

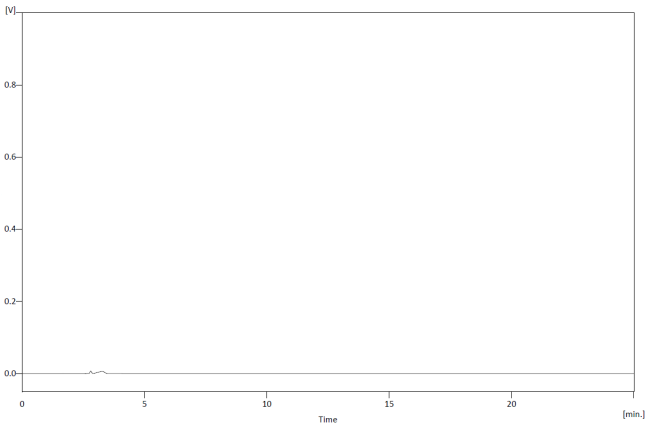

Fig. 5: Blank Chromatogram

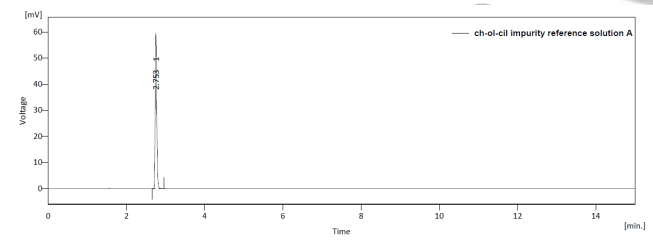

Fig. 6: Chlorthalidone Impurity-A standard chromatogram

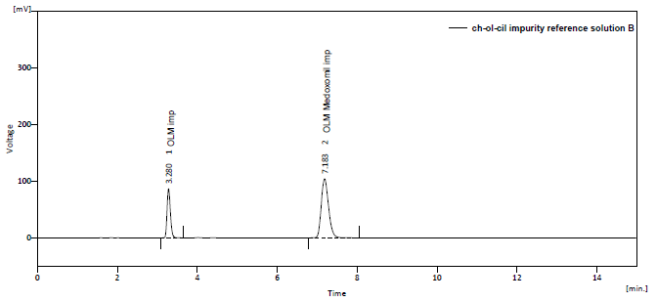

Fig. 7: Olmesartan and Olmesartan Medoxomil Impurity-A standard Chromatogram

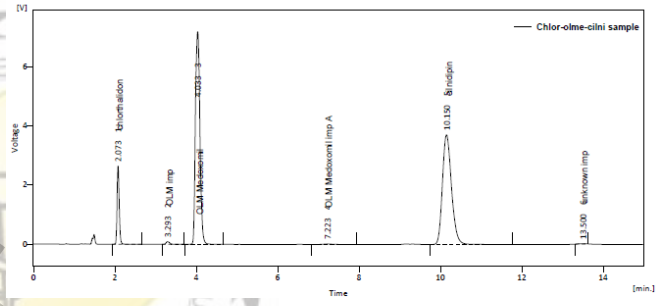

Fig. 8: Sample chromatogram

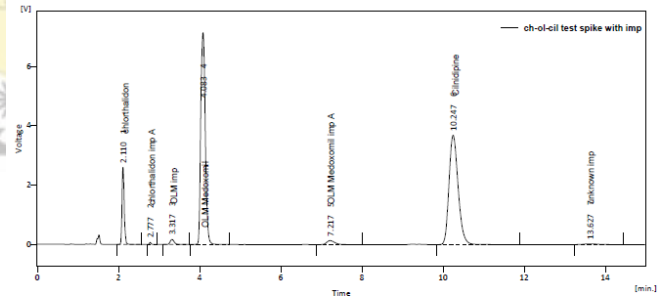

Fig. 9: Chromatogram of Sample spiked with known impurities

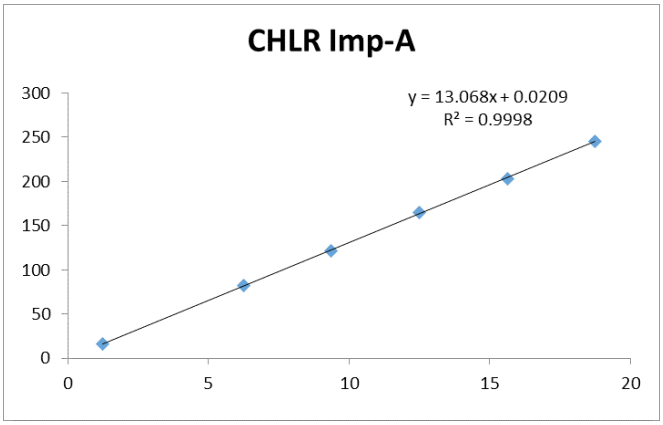

Fig. 10: Calibration Curve of Chlorthalidone Impurity-A

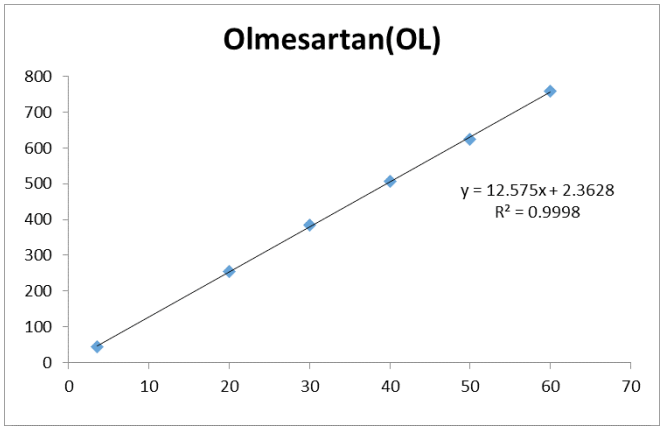

Fig. 11 Calibration Curve of Olmesartan

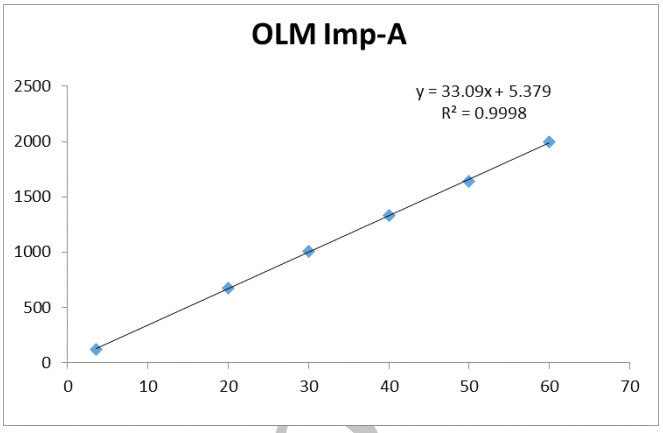

Fig. 12: Calibration Curve of Olmesartan Medoxomil Impurity-A

The results presented an LoD and LoQ for Chlorthalidone Impurity-A of 0.4 and 1.2 µg/mL, Olmesartan of 1.2 µg/mL and 3.5 µg/mL, Olmesartan Medoxomil Impurity-A 1.1 µg/mL and 3.3 µg/mL respectively.

Accuracy

The accuracy of an analytical procedure describes the closeness to the accurate value generated by a method. The results of accuracy expressed in % recovery at all four levels in the range of 97.4–101.4%, and RSD (%) values were in the range of 0.64–2.1% for Chlorthalidone Impurity-A, 91.3–102.9%, and RSD (%) values were in range of 1.06–4.63% for Olmesartan, 95.9–102.0%, and RSD (%) values were in range of 0.64–2.56% for Olmesartan Medoxomil Impurity-A shown in Table 4-7. The results of recovery (%) were within accepted limits from 90.0% to 110.0% for 80%, 100% and 120%, from 70.0% to 130.0% for LOQ level respectively. The results of percentage RSD were within the accepted limits below 10.0% for 80%, 100%, and 120%, below 15.0% for LOQ level, respectively. This proves its validating of the method for routine drug analysis.

Precision

The precision of the method is derived as "the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is generally

Table 4: Sample for Recovery (As such)

Sr. No	*Recovery sample Chlr Imp-A Area*	*OL Area*	*OLM imp-A Area*
1	Not present	547.864	272.007
2	Not present	553.897	274.983
3	Not present	548.302	266.104
Avg	-	550.021	271.031
SD	-	3.364	4.519
%RSD	-	0.612	1.667

Table 5: Accuracy results for Chlorthalidone Impurity-A

Level	*Added Amount (µg/ml)*	*Recovered Amount (µg/ml)*	*recovery%*	*% Avg.*	*SD*	*%RSD*
LOQ	1.25	1.257	100.533	99.7	2.090	2.095
LOQ	1.25	1.267	101.328			
LOQ	1.25	1.217	97.377			
80%	10.0	9.851	98.513	98.7	0.988	1.001
80%	10.0	9.979	99.789			
80%	10.0	9.784	97.844			
100%	12.5	12.520	100.158	100.6	0.646	0.642
100%	12.5	12.672	101.377			
100%	12.5	12.550	100.398			
120%	15.0	14.982	99.881	100.6	0.721	0.717
120%	15.0	15.198	101.321			
120%	15.0	15.079	100.524			

Table 6: Accuracy results for Olmesartan

Level	*Added Amount (µg/ml)*	*Recovered Amount (µg/ml)*	*recovery%*	*% Avg.*	*SD*	*%RSD*
LOQ	3.6	3.426	95.156	95.5	4.425	4.632
LOQ	3.6	3.605	100.139			
LOQ	3.6	3.287	91.313			
80%	32.0	32.272	100.851	101.1	1.300	1.286
80%	32.0	31.966	99.893			
80%	32.0	32.789	102.465			
100%	40.0	40.170	100.426	101.7	1.263	1.241
100%	40.0	40.751	101.877			
100%	40.0	41.177	102.942			
120%	48.0	48.431	100.898	101.6	1.075	1.057
120%	48.0	49.377	102.868			
120%	48.0	48.547	101.139			

Table 7: Accuracy results for Olmesartan Medoxomil Impurity-A

OLM Imp.-A						
Level	*Added Amount (µg/ml)*	*Recovered Amount (µg/ml)*	*recovery%*	*% Avg.*	*SD*	*%RSD*
LOQ	3.6	3.627	100.741			
LOQ	3.6	3.590	99.726	98.8	2.530	2.560
LOQ	3.6	3.454	95.941			
80%	32.0	32.130	100.407			
80%	32.0	31.970	99.907	100.5	0.714	0.710
80%	32.0	32.421	101.314			
100%	40.0	39.987	99.967			
100%	40.0	40.319	100.798	100.9	1.033	1.023
100%	40.0	40.808	102.020			
120%	48.0	48.272	100.566			
120%	48.0	48.836	101.741	101.0	0.649	0.643
120%	48.0	48.324	100.676			

expressed as the RSD. Based on the results of both systems and methods, precision proved that the method is precise within satisfactory limits. The tailing factor, RSD, and theoretical plats were determined, and all the results are within acceptance criteria. Acceptable precision was less than 2.0 the tailing factor, NMT 10.0% for the RSD and NLT 2000 for a number of plates, as reported in Tables 8-11.

Robustness

Robustness was evaluated for an analytical method by assessing the influence of minor changes in chromatographic conditions on system suitability parameters and % impurity value difference from as such condition of the proposed method. The results of robustness testing proved that minor deliberate changes

Table 8: System Precision

System precision			
Sr. No	*Chlr Imp.A*	*OL*	*OLM Imp-A*
	Area		
1	164.851	499.064	1306.927
2	163.687	504.577	1327.310
3	165.492	509.624	1340.638
4	167.321	514.223	1352.721
5	165.985	509.067	1339.137
Avg.	165.467	507.311	1333.347
SD	1.347	5.738	17.295
%RSD	0.814	1.131	1.297

Table 9: Method Precision

	OL		*OLM-imp A*		*Unknown imp*	
Sr. No.	*Area*	*%RS*	*Area*	*%RS*	*Area*	*%RS*
1	548.480	1.081	271.006	0.203	95.181	0.078
2	544.641	1.074	269.009	0.202	82.753	0.074
3	540.106	1.065	266.944	0.200	92.022	0.076
4	533.658	1.052	263.816	0.198	90.688	0.076
5	538.877	1.062	266.474	0.200	93.536	0.078
Avg.	-	1.067	-	0.201	-	0.076
SD	-	0.011	-	0.002	-	0.002
%RSD	-	1.047	-	1.015	-	2.190

Table 10: Intermediate Precision

Reproducibility						
	OL		*OLM-imp A*		*Unknown imp*	
Sr.no	*Area*	*%RS*	*Area*	*%RS*	*Area*	*%RS*
1	543.206	1.053	268.485	0.198	93.325	0.077
2	540.901	1.049	267.092	0.197	93.779	0.077
3	545.760	1.058	269.595	0.199	87.302	0.071
4	539.287	1.046	266.594	0.197	83.377	0.069
5	545.197	1.057	267.693	0.198	87.597	0.072
Avg.	-	1.053	-	0.198	-	0.073
SD	-	0.005	-	0.001	-	0.004
%RSD	-	0.509	-	0.442	-	4.963

in method conditions, eg. Flow rate, mobile composition, and pH of the buffer is robust within the acceptable criteria. The results are summarized in Table 12-15. In all modifications, system suitability was achieved and % Impurity value was observed well within acceptable limits as well.

Table 11: Overall Precision (Method and Intermediate Precision)

Overall Precision			
	OL	*OLM-Imp A*	*Unknown imp*
Sr.no	*%RS*	*%RS*	*%RS*
1	1.081	0.203	0.078
2	1.074	0.202	0.074
3	1.065	0.200	0.076
4	1.052	0.198	0.076
5	1.062	0.200	0.078
6	1.053	0.198	0.077
7	1.049	0.197	0.077
8	1.058	0.199	0.071
9	1.046	0.197	0.069
10	1.057	0.198	0.072
Avg.	1.060	0.199	0.075
SD	0.011	0.002	0.003
%RSD	1.04	1.06	4.22

Table 12: System suitability for Variation in Flow rate, Organic solvent & pH

System Suitability	*Results*
Flow Rate (+0.2) & (-0.2)	complies
Organic Solvent (+2 ml) & (-2 ml)	complies
pH (+0.2) & (-0.2)	complies

Table 13: Comparison with Method Precision

FR +0.2			
Mean value of Impurity	*OL (%)*	*OLM Imp. A (%)*	*Un-known Imp. (%)*
As per Method	1.067	0.201	0.076
Flow rate (+0.2 ml)	1.112	0.210	0.090
% Diff.	0.045	0.009	0.014
Result	Complies	Complies	Complies
FR -0.2			
Flow rate (-0.2 ml)	1.066	0.200	0.060
% Diff.	0.001	0.001	0.016
Result	Complies	Complies	Complies

Table 14: Comparison with Method Precision

Organic Solvent +2 ml			
Mean value of Impurity	*OL (%)*	*OLM Imp. A (%)*	*Un-known Imp. (%)*
As per Method	1.067	0.201	0.076
Organic Solvent (+2 ml)	1.094	0.206	0.076
% Diff.	0.027	0.005	0.000
Result	Complies	Complies	Complies
Organic Solvent -2 ml			
Organic Solvent (-2 ml)	1.086	0.204	0.078
% Diff.	0.019	0.003	0.002
Result	Complies	Complies	Complies

Table 15: Comparison with Method Precision

pH +0.2			
Mean value of Impurity	*OL (%)*	*OLM Imp. A (%)*	*Un-known Imp. (%)*
As per Method	1.067	0.201	0.076
pH (+0.2)	1.086	0.204	0.078
% Diff.	0.019	0.003	0.002
Result	Complies	Complies	Complies
pH -0.2			
pH (-0.2)	1.087	0.203	0.070
% Diff.	0.02	0.002	0.006
Result	Complies	Complies	Complies

Conclusion

In the described research, a simple, fast, accurate, precise, and linear stability-indicating analytical method has been developed and validated for Related Impurities of Olmesartan Medoxomil, Chlorthalidone, and Cilnidipine drug combinations. Hence, it can be further employed for quality control routine analysis. The analytical method conditions and mobile phase provided a good resolution for all peaks of an analyte. In addition, the main advantage of the developed method is with less run time. The method was further validated as per ICH guidelines. The method is robust enough to reproduce precise and accurate results under varied chromatographic conditions.

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