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Research Article

Related Impurities High-performance Liquid Chromatography Method Development and Validation for drug combinations: Olmesartan Medoxomil, Chlorthalidone and Cilnidipine

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ABSTRACT

The liquid chromatography mass spectrometry (LC-MS) compatible, stability-indicating, specific, linear, accurate, sensitive with less run-time related impurities reversed phase high-performance liquid chromatography (RP-HPLC) related impurities method has been developed for olmesartan medoxomil (OLM), chlorthalidone (CHLR), and cilnidipine (CIL) 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, C18 (12.5 cm, 4.6 mm, 5 microns particle size) column. Mobile phase-A 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 CHLR impurity A, olmesartan (OL), OLM 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 limit of quantitation (LoQ)–18.75 µg/mL, 3.6 LoQ–60.0 µg/mL, 3.6 LoQ–60.0 µg/mL respectively for CHLR impurity A, OL and OLM impurity A respectively. The limit of detection (LoD) and LoQ were found 0.4 ppm (µg/mL) and 1.2 ppm (µg/mL), 1.2 ppm (µg/mL) and 3.5 ppm (µg/mL), 1.1 ppm (µg/mL) and 3.3 ppm (µg/mL) for CHLR impurity A, OL and OLM 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 OLM, CHLR, and CIL drug combinations.

Introduction

Olmesartan medoxomil (OLM) is a synthetic imidazole derivative pro-drug with an antihypertensive property (Fig. 1). The OLM prevents angiotensin II induced vasoconstriction and decreases aldosterone production, thereby preventing aldosterone-stimulated sodium retention and potassium excretion. Chlorthalidone (CHLR) is a diuretic medication used to treat high blood pressure, swelling including that due to heart failure, liver failure and

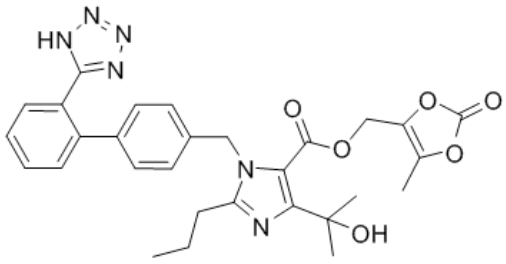

Fig. 1: Chemical structure of Olmesartan Medoxomil

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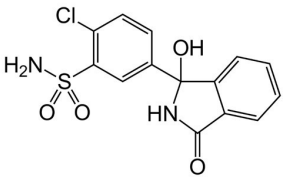

Fig. 2: Chemical structure of Chlorthalidone

nephrotic syndrome, diabetes insipidus, and renal tubular acidosis. (Fig. 2). CIL is a calcium channel blocker. CIL decreases blood pressure and is used to treat hypertension and its comorbidities. OLM, CHLR and CIL combinations are used to treat hypertension when a single medication is not effective (Fig. 3). It also helps to reduce chances of future heart attack and stroke. The most important related compounds for OLM are OL and OLM impurity-A, for CHLR is CHLR impurity-A. A literature survey discloses that few stability-indicating HPLC methods,[1-18] HPTLC,[19-20] Spectrophotometric methods[21-22] have been reported for the estimation of OLM and or CHLR and or CIL along with drug combinations in pharmaceutical preparations. To the best to our knowledge, no reports were found for stability-indicating LC-MS compatible related impurities method for OLM, CHLR and CIL 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 (OL, OLM impurity-A, CHLR impurity-A, and un-known impurities) for OLM, CHLR and CIL 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.

Materials and Methods

Materials

A pharmaceutical-grade gift sample of OLM (established purity 99.2%), CHLR (purity 98.8%), CIL (purity 99.5%) were acquired from Amoli Organics Pvt Ltd. Olkem Trio 40 tablets containing OLM 40 mg, CHLR 12.5 mg and CIL 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.

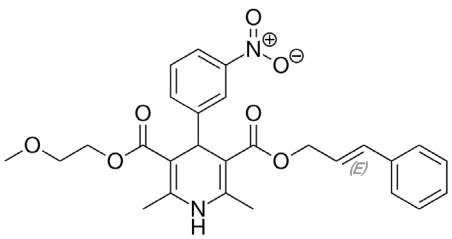

Fig. 3: Chemical structure of Cilnidipine

Methods

Instrumentation

The LC-20AT (Shimadzu) system was used for HPLC method development and validation by using Hypersil BDS, C18 (12.5 cm × 0.46 cm) 5 microns 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 microns 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)

Preparation of Standard Solution

- CHLR impurity-A stock solution (125 µg/mL): Weigh accurately about 12.5 mg of CHLR impurity-A and transfer to a 100 mL volumetric flask. Add 60 mL methanol, sonicate till dissolve and make up the volume up to the mark with methanol.
- OL stock solution (400 µg/mL): Weigh accurately about 40 mg of OL and transfer to a 100 mL volumetric flask. Add around 60 mL methanol, sonicate to dissolve, and make up the volume up to the mark with methanol.
- OLM impurity-A stock solution (400 µg/mL): Weigh accurately about 40 mg of OLM impurity-A and transfer into a 100 mL 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 (12.5 µg/mL), OL (40 µg/mL) and OLM impurity-A (40 µg/mL): Take 1 mL CHLR impurity-A stock solution, 1 mL OL stock solution and 1 mL 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.

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

- CHLR standard stock solution (125 µg/mL): Weigh accurately about 12.5 mg of CHLR and transfer to a 100 mL volumetric flask. Add 60 mL methanol, sonicate till dissolve, and makeup volume up to the mark with methanol.
- OLM standard stock solution (400 µg/mL): Weigh accurately about 40 mg of OLM and transfer in 100 mL volumetric flask. Add about 60 mL methanol, sonicate to dissolve, and makeup volume up to the mark with methanol.
- CIL standard stock solution (100 µg/mL): Weigh accurately about 10 mg of CIL and transfer in a 100 mL 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 (12.5 µg/mL), OLM (40 µg/mL) and CIL (10 µg/mL): Take 1 mL CHLR stock solution, 1 mL OLM stock solution, and 1 mL 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 10 mL volumetric flask. Add 8 mL diluent, shake for 15 minutes and sonicate the solution for 10 minutes. Make up the volume with diluent and mix well to obtain OLM (4000 µg/mL), CHLR (1250 µg/mL), and CIL (1000 µg/mL). Filter this solution with a 0.45 µm 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 OL impurity, OLM Impurity-A and CHLR 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 and LoQ of OL impurity, OLM impurity-A, and CHLR impurity-A were performed by preparing different solutions of OL impurity, OLM impurity-A, and CHLR 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 OL impurity, OLM impurity-A, and CHLR impurity-A and triplicate samples for individual concentration were injected. The recovery (%) for added OL impurity, OLM impurity-A and CHLR impurity-A and RSD were measured for individual replicate samples.

Precision

The system precision and repeatability (method precision) for proposed methods were performed by multiple measurements of standard and 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 OLM, CHLR and CIL drug combinations. A Hypersil BDS, C18 (12.5 cm × 0.46 cm) 5 microns column was selected as the stationary phase for the separation and determination of related impurities method for OLM, CHLR and CIL 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 Table 3.

Table 2: Method development summary

S. 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.

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

Method was optimized with flow rate of 1.0 mL/minutes, wavelength 260 nm, 20 µL volume of injection and 25.0°C column temperature as the best chromatographic conditions for the complete study where OLM, CHLR, CIL, OL impurity, OLM Impurity-A and CHLR 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 (OLM, CHLR and CIL), impurity standard (OL and OLM impurity-A, CHLR impurity-A), as such sample and sample spiked with OL, OLM impurity-A and CHLR 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 OL, OLM impurity-A and CHLR impurity-A impurities sample solution were injected into the HPLC system individually, and the chromatogram are shown in Figs. 5–9. It can be observed that there no co-eluting peaks at the retention time of OLM, CHLR, CIL, OL, OLM impurity-A and CHLR 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

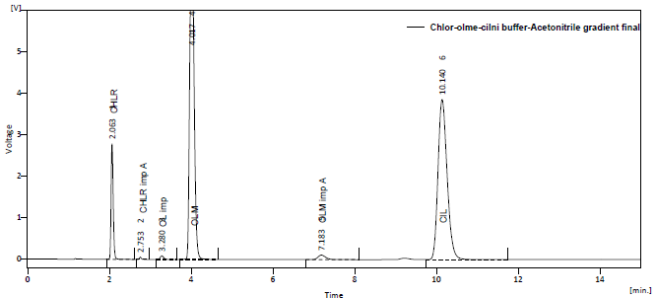

Fig. 4: Sample (spiked) chromatogram

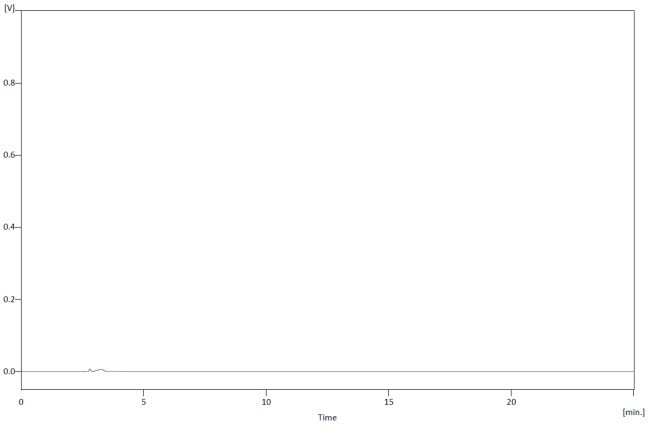

Fig. 5: Blank chromatogram

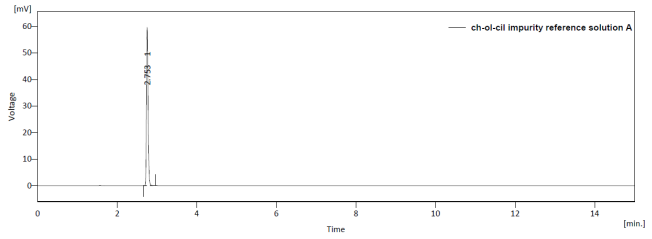

Fig. 6: Chlorthalidone impurity-A standard chromatogram

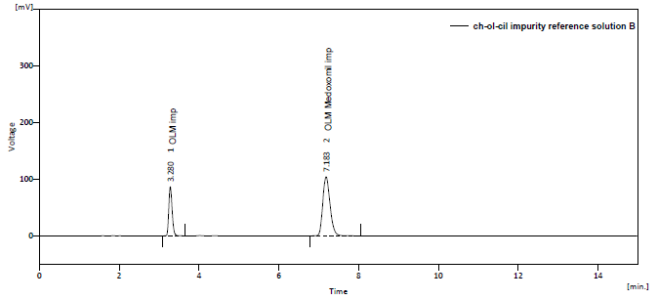

Fig. 7: OL and OLM impurity-A standard Chromatogram

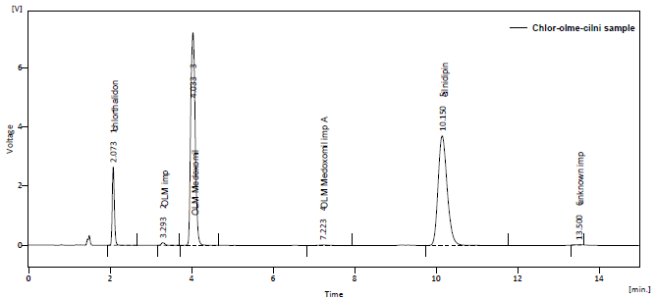

Fig. 8: Sample chromatogram

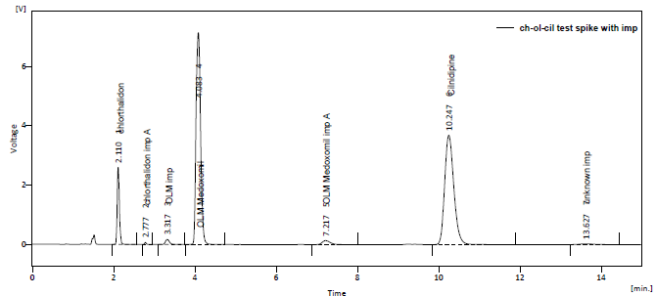

Fig. 9: Chromatogram of sample spiked with known impurities

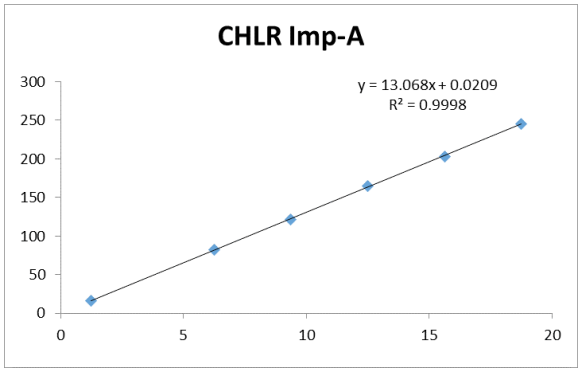

Fig. 10: Calibration curve of chlorthalidone impurity-A

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 (1.25 µg/mL-18.75 µg/mL), LoQ (3.6 µg/mL-60 µg/mL) and LoQ (3.6 µg/mL-60 µg/mL) respectively. Based on regression calculation, a linear equation was obtained: $y = mx + c$, and r^2 was found greater than 0.990, representative a linear relationship for the concentration of analytes and peak area (Figs. 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. The results presented an LoD and LoQ for CHLR impurity-A of 0.4 and 1.2 µg/mL, OL of 1.2 µg/mL and 3.5 µg/mL, OLM 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 CHLR impurity-A, 91.3–102.9%, and RSD (%) values were in range of 1.06–4.63% for OL, 95.9–102.0%, and RSD (%) values were in range of 0.64–2.56% for OLM impurity-A shown in

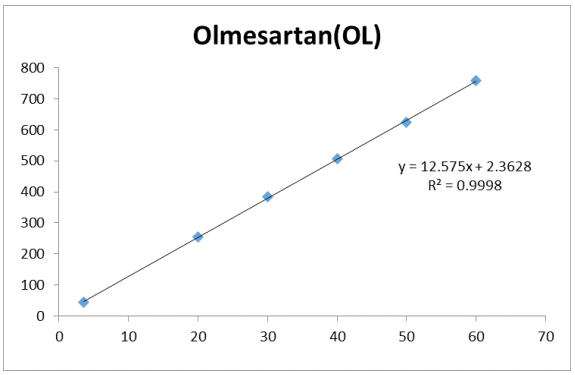

Fig. 11: Calibration curve of olmesartan

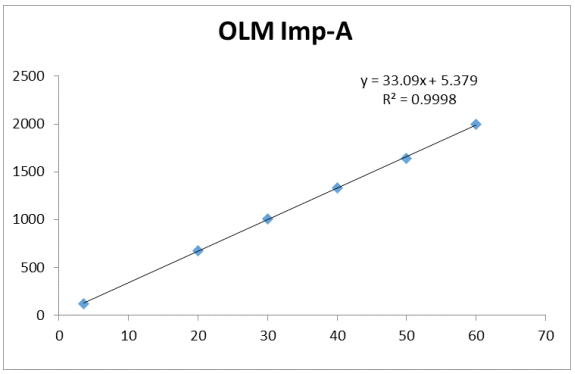

Fig. 12: Calibration curve of OLM impurity-A

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 expressed as the RSD. Based on the results of both systems and method 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.

Table 4: Sample for recovery (as such)

	Recovery sample		
	CHLR imp-A	*OL*	*OLM imp-A*
S. No.	*Area*	*Area*	*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 OLM 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			

Table 8: System precision

System precision			
S. 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*	
S. 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*	
S. 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

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 in method conditions, eg. flow rate, mobile composition, and pH of the buffer is robust within the acceptable criteria. The results are summarized in Tables 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*
S. 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 and pH

System suitability	*Results*
Flow rate (+0.2) and (-0.2)	Complies
Organic solvent (+2 mL) and (-2 mL)	Complies
pH (+0.2) and (-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 OLM, CHLR, and CIL 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.

References

1. Sidhdhapara MJ, Patel B, Paramar A. Development and validation of RP-HPLC method for simultaneous estimation of Cilnidipine and OLM in their combined tablet dosage form. International Journal of Pharmacy and Biological Sciences. 2014; 4(1): 157-160.
2. Nayak PR, Chaudhary AB, Rahevar NM. RP-HPLC method development and validation for simultaneous estimation of Chlorthalidone, Cilnidipine and OL in tablet dosage form. World Journal of Pharmacy and Pharmaceutical Sciences. 2016; 5(6): 1498-1508.
3. Vartak JP, Roy SMN. Simultaneous determination of Clinidipine, OLM and Chlorthalidone in pharmaceutical preparations using validated, LCMS compatible RP-HPLC method. Analytical Chemistry An Indian Journal. 2015; 15(3): 105-110.
4. Patel RD, Luhar SV, Narkhede SB. Analytical method development and validation for simultaneous estimation of Cilnidipine, OL and Chlorthalidone in synthetic mixture by RP-HPLC method. Journal of Pharmaceutical Science and Bio-scientific Research. 2016; 6(3): 308-314.
5. Chinthala K, Krishnamurthy M, Kumar P. Stability indicating method development and validation for the simultaneous estimation of Olmesartan, chlortalidone and Cilnidipine in bulk and pharmaceutical dosage form by using RP-HPLC. International Journal of Pharmacy. 2016; 6(3): 149-160.
6. Sunitha N, Marihal SC, Sravanthi JS, Venu A, Rao BVN, Rao BA. Method development and validation of RP-HPLC method for the simultaneous estimation of OL and Cilnidipine in bulk and formulations. International Journal of Pharmaceutical Research and Allied Sciences. 2015; 4(3): 127-135.
7. Bapna M, Bhai KG. Development and validation of analytical method for simultaneous estimation of Cilnidipine and Chlorthalidone in their combined dosage form. International Journal Ayu. Pharm Chem. 2015; 2(3): 51-58.
8. Kudumula N, Prasad YR. Development and validation of RP-HPLC method for the simultaneous estimation of Chlorthalidone and Cilnidipine in bulk and combined tablet dosage form. Pharmacophore. 2014; 5(4): 442-450.
9. Patel MP, Patel KP, Patel DB. Development and validation of analytical method for simultaneous estimation of Cilnidipine, Chlorthalidone and Telmisartan in tablet dosage form. World Journal of Pharmacy and Pharmaceutical Sciences. 2016; 5(6): 1228-1241.
10. Prasad KC, Rama Krishna M, Jeevanmani Babu D, Kumar NA, Babu GR. Stability-indicating simultaneous estimation of Metoprolol and Cilnidipine by using RP-HPLC. World Journal of Pharmacy and Pharmaceutical Sciences. 2017; 6(1): 1395-1403.
11. Yadav SP, Sowjanya P, Kumar GV. Development of validated stability indicating assay method by RP-HPLC for simultaneous estimation of Azilsartan medoximil and Chlorthalidone in their bulk and combination form. International Journal of Medicine and Pharmaceutical Research. 2017; 5(6): 182-186.
12. Kashyap R, Srinivasa U. Development and validation of HPLC method for the simultaneous estimation of Chlorthalidone and Metoprolol succinate in bulk and dosage form. International Journal of Pharmaceutics and Drug Analysis 2013; 1(2): 1-14.
13. Patel PN, Prajapati AP, Luhar SV, Narhede SB. Stability indicating RP-HPLC method for determination of Atenolol and Chlorthalidone in pharmaceutical dosage form. European Journal of Biomedical and Pharmaceutical Sciences. 2018; 5(5): 708-715.
14. Pawar VT, Pawar SV, More HN, Kulkarni AS, Gaikwad DT. RP-HPLC method for simultaneous estimation of Cilnidipine and Chlorthalidone. Research J. Pharm. And Tech. 2017; 10(11): 3990-3996.
15. Aruna G, Bharathi K, Prasad K. Development and validation of bio-analytical HPLC Method for simultaneous estimation of Cilnidipine and Nebivolol in human plasma. Int. J Pharm Pharm. Sci. 2017; 9(10): 253-259.
16. Rupareliya RH, Joshi HS. Stability indicating simultaneous validation of Telmisartan and Cilnidipine with forced degradation behavior study by RP-HPLC in tablet dosage form. International Scholarly Research Notices Chromatography. 2013; article ID 461461.
17. Kasimala MB, Kasimala BB. Reverse phase-HPLC method development and validation for the simultaneous estimation of Azilsartan medoxomil and Chlorthalidone in pharmaceutical dosage forms. Journal of Atoms and Molecules. 2012; 2(1): 117-126.
18. Kachave R, Kale M, Wagh RD. Simultaneous estimation of Cilnidipine and Valsartan by RP-HPLC in tablet formulation. Eurasian Journal of Analytical Chemistry. 2016; 11(5): 245-253.
19. Sangle S, Deshpande P, Shinde N, Tayade V. Development and validation of stability indicating HPTLC method for simultaneous determination of OLM and Chlorthalidone in combined tablet dosage forms. European Journal of Pharmaceutical Medical Research. 2017; 4(7): 574-581.
20. Butle SR, Deshpande PB. Development and validation of stability-Indicating HPTLC method for simultaneous determination of Telmisartan and Cilnidipine in combined tablet dosage form. International Journal of Pharmaceutical Sciences and Drug Research. 2015; 7(6): 478-483.

21. Reddy BH, Spandana B, Mounika D, Devi S, Dacha A, Prakash KV. Simultaneous estimation of Telmisartan, Chlorthalidone and Cilnidipine by absorbance correction method using UV spectrophotometry. Indo American Journal of Pharmaceutical Sciences. 2018; 05(03): 1998-2003.
22. Jani RJ, Patel S. Development and validation of Spectrophotometric method for simultaneous estimation of Azilsartan Kamedoxomil and Cilnidipine in synthetic mixture. World Journal of Pharmaceutical Research. 2018; 3(2): 86-90.
23. International Conference on Harmonization (2005) ICH harmonized tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva. Nov. (2005).
24. International Conference on Harmonization (ICH): Stability testing of new drug substances and products. Q1A (R2), (2003).

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