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Research Article

Analytical Study and Impurity Profiling of Fixed Doses Combination of Amlodipine, Hydrochlorothiazide and Olmesartan by RP-HPLC and UPLC

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ABSTRACT

A specific and sensitive reverse phase high pressure liquide chromatography (RP-HPLC) and ultra-performance liquide chromatography (UPLC) method for the determination of related substances for fixed-dose combination formulation of Amlodipine Besylate, Hydrochlorothiazide and Olmesartan Medoxomil was developed and validated. By using the gradient RP-HPLC method, a total of 25 known and unknown impurities related to Amlodipine Besylate, Hydrochlorothiazide and Olmesartan Medoxomil in combination formulation were separated and quantified in the linearity range of (0.2 to 3.0 µg/mL) for Amlodipine Besylate, (0.5 to 7.5 µg/mL) for Hydrochlorothiazide and (0.8 to 12.0 µg/mL) for Olmesartan Medoxomil with good squared correlation (>0.99). The recovery study was established from LOQ (0.05%) to 150% of the specification limit. A precision study was performed by spiking each known impurities at the specification level. In this study, two separate analytical methods RP-HPLC and UPLC have been developed, validated, and compare for the simultaneous quantification of Amlodipine Besylate, Hydrochlorothiazide, and Olmesartan Medoxomil tablets with their impurities. In these methods total of 28 peaks were separated in 70 minutes run time by HPLC method while in 45 minutes by UPLC method. Both the methods are developed with high selectivity, sensitivity, and robustness to separate 28 peaks in a single method. The proposed methods have a high degree of sensitivity and will provide fast and cost-effective quantitative control of Pharmaceutical formulations.

Introduction

Hypertension or elevated blood pressure is a serious medical condition that involves a higher risk of heart, brain, kidney, and other diseases. Worldwide major population is suffering from a heart-related disease directly in correlation with hypertension. No treatment or late treatment of hypertension can lead to the cause of illness or death. Early diagnosis and treatment of hypertension can prevent heart, brain, and chronic kidney disease. Many products are available to cure hypertension as a single drug formulation and fixed-dose combination product, but fixed-dose combination products are more efficient in treating hypertension as per various research. A triple-drug combination of Amlodipine Besylate (AMD), Hydrochlorothiazide (HCTZ), and Olmesartan Medoxomil (OLM) is one of the fixed-dose combinations which are very effective and widely used products for the treatment of hypertension. Various researches show that this triple combination is useful for significantly controlling cardiac activity and lowering the risk of coronary cardiac disease and other critical conditions related to the brain and kidney.[1-4]

The fixed-dose combination for AMD,[5] HCTZ,[6] and OLM[7] is available in the market under various brands. Many researchers have reported determining impurities related to all three active pharmaceutical ingredients (API) by RP-HPLC for this triple combination product with a longer run time. However, no research is reported

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to separate all process and degradation impurities of AMD, HCTZ, and OLM by a single method.[8-10] Impurity profiling with separation and identification of each related impurities of individual APIs in combination products is very critical, and it is more important for safety assessment and formulation process development.

Literature survey shows many analytical methods for assay methods are reported simultaneous estimation of HCTZ, AMD, and OLM by RP-HPLC and UPLC. Some of the methods were reported in a combination of other drugs by HPLC and UPLC. Mali AD *et al*. have reported impurity profiling for a combination of OLM and HCTZ by RP-HPLC method. In this research work, impurities related to AMD were not evaluated.[11] Prasad VD. *et al*. and Desai PR. *et al*. have reported impurity profiling of AMD, HCTZ and OLM by RP-HPLC in their respective research work. However, many impurities of the individual drug were identified and reported in individual research work, but all reported impurities of each drug were not included in reported research.[12-13] Many research works have reported impurity profiling for individual drugs and the combination of other drugs. Some of the research work has been done on advanced analytical separation techniques like HPLC and UPLC.[14-26] However, research reported by various researchers is very limited. In this research work, a single chromatographic method was developed by RP-HPLC to separate all possible degradation products and process impurities of each API and validate them according to ICH guidelines. Additionally, a separate method with a concise run time is developed by UPLC for the separation of all possible degradation, and process impurities related to each API and an equivalency study between HPLC and UPLC method was performed.

The study is important for separating and quantifying all possible degradation and process-related impurities of AMD, HCTZ and OLM in the fixed-dose combination products in a single chromatographic method. It helps to identify and control any degradation product in formulation with a safe and good approach for the product life cycle. The structures of AMD, HCTZ, OLM and related impurities of these drugs are demonstrated in Figs 1-6.

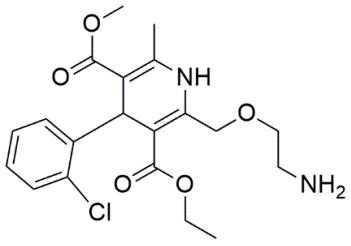


Fig. 1: Chemical structure of AMD

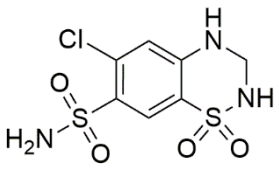


Fig. 2: Chemical structure of HCTZ

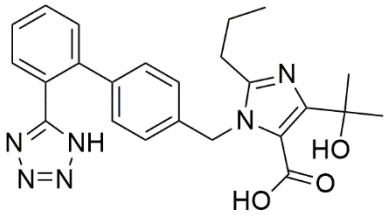


Fig. 3: Chemical structure of OLM

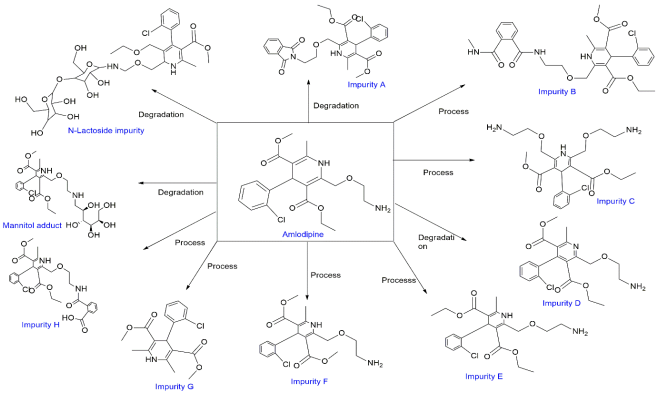


Fig. 4: Chemical structure of AMD and its impurities

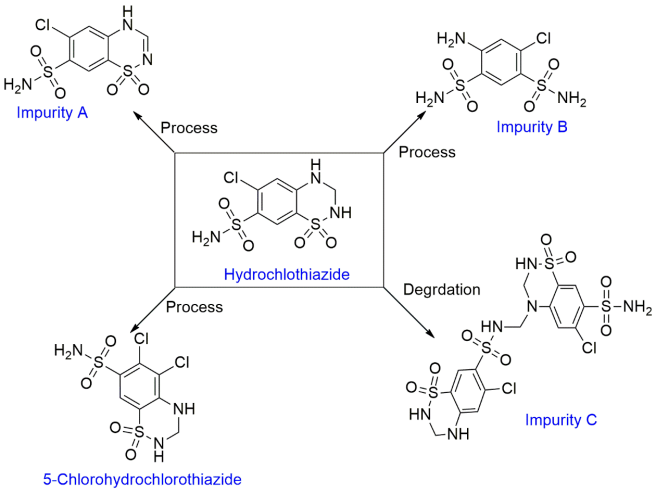


Fig. 5: Chemical structure of HCTZ and its impurities

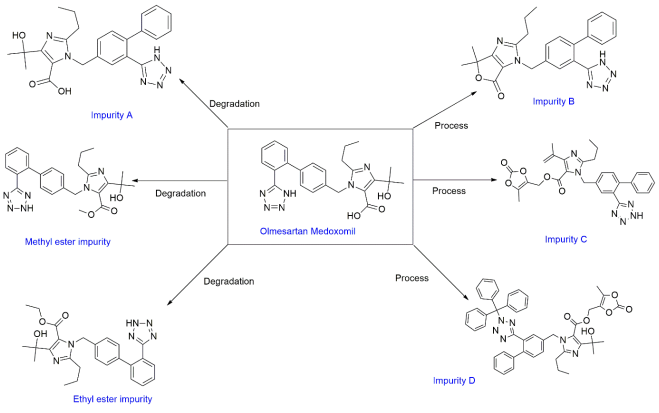


Fig. 6: Chemical structure of OLM and its impurities

In this research, two separate methods were developed by HPLC and UPLC. Equivalency between both methods is proved. These methods can be adopted in pharmaceutical industries for routine analysis to save time and project costs. Industries having facility of UPLC instrument can be adopted UPLC method from this research work. The shorter run time of the UPLC and HPLC method with a high degree of selectivity and sensitivity is a big advantage for Pharmaceutical industries.

Material and Method

Material and Reagents

AMD API, HCTZ API, OLM API, related known impurities were received from Paradise Healthcare and combination tablets of AMD, HCTZ and OLM were purchased from the market. HPLC grade water, Acetonitrile, potassium dihydrogen phosphate and orthophosphoric acid were purchased from Merck.

Solution Preparation

10 mM Phosphate Buffer pH 3.0

Weighed 2.72gm of potassium dihydrogen phosphate (KH_2PO_4) and dissolved it in 1000 mL of water.

Carefully pH was adjusted to 3.0 ± 0.05 with the dilute ortho-phosphoric acid solution. Mixed well and filter through a 0.45µm membrane filter before use.

Mobile phase-A

10 mM phosphate buffer pH 3.0: Acetonitrile (95:5%v/v).

Mobile Phase B

10 mM phosphate buffer pH 3.0: Acetonitrile (10:90%v/v).

Diluent Preparation

Diluent was prepared by mixing water (400 mL) and acetonitrile (600 mL).

Sample Solution Preparation

Weigh 20 tablets and the average weight was determined. Tablets were crushed by mortar-pastel to a fine powder and weigh accurately sample powder quantity equivalent to 10.0 mg of AMD, transfer into 25 mL volumetric flask, added 15 mL of diluent and sonicate for 20minutes with intermediate shaking, diluted it to volume with diluent. Mixed well and filtered the clear supernatant solution through 0.45 µm syringe filter and analyzed in HPLC system.

High-Performance Liquid Chromatography (HPLC analytical)

A Waters HPLC system with a quaternary pump, photo-diode array detector, and auto-sampler has been used for analysis. An RP-HPLC gradient method is used to determine known and unknown impurities and degradation products related to AMD, HCTZ, and OLM combination tablets. A Waters X-Bridge Phenyl (4.6X150 mm) 3.0 µ column was used for analysis with a flow rate of 0.9 mL/minutes. The analyte peaks were monitored, and the detection wavelength was 237 nm. A mixture of 10mM phosphate buffer pH 3.0 and acetonitrile in the ratio of 95:5 %v/v was used as mobile phase-A. Prepared 10mM phosphate buffer pH 3.0±0.05 and mixed with acetonitrile in the ratio of 10:90 %v/v and used as a mobile phase-B. The mobile phase gradient was started at 10% of B up to 3.00 minutes and increased to 40% of B up to 35 minutes then increased to 80% of B up to55 minutes then decreased to 60% of B up to 58 minutes then further decrease to 50% of B up to 62 minutes then started initial gradient ratio from 62.1 minutes for gradient saturation up to 70 minutes. The injection volume was 20 µL. Column temperature 30°C.

Diluent prepared by proper mixing of water and acetonitrile in the ratio of 40:60%v/v.

Ultra-performance liquid chromatography (UPLC)

A separate method was developed to determine known and unknown impurities and degradation products related to AMD, HCTZ and OLM by the UPLC technique. The Waters H-Class UPLC system separated all possible degradation products and impurities associated with AMD, HCTZ and OLM combination products. A Waters Acquity UPLC BEH Phenyl (2.1X75 mm), 1.7 µ column was used for analysis with a flow rate of 0.3 ml/minutes. The analyte peaks were monitored and the detection wavelength was 237 nm. A mixture of 10 mM phosphate buffer pH 3.0 and acetonitrile in the ratio of 95:5 %v/v was used as mobile phase-A. Prepared 10 mM phosphate buffer pH 3.0±0.05 and mixed with acetonitrile in the ratio of 10:90 %v/v and used as a mobile phase-B.

The mobile phase gradient was started at 5% of B and increased to 25% B up to 10 minutes then increased to 70% of B up to25 minutes then increased up to 90% up to 35 minutes and from 35.1 minutes initial gradient for saturation up to 45 minutes. The injection volume was 3 µL. Column temperature 30°C. Diluent prepared by proper mixing of water and acetonitrile in the ratio of 40:60 %v/v.

Method Validation

The method for determination of impurities and degradation products related to AMD, HCTZ and OLM by HPLC was validated as per ICH guidelines with all validation parameters like specificity, LOD, and LOQ determination, linearity range, repeatability, accuracy, and robustness are included in the study. An optimized method by UPLC and verified by performing Specificity and LOQ precision. An equivalency study between HPLC and UPLC method was performed.

Specificity (By HPLC and UPLC)

Specificity is the ability to assess the analyte unequivocally in the presence of components expected to be present. Typically, these might include impurities degradation

products. The specificity of the method was performed by injecting diluent solutions, individual known impurities, sample solutions individually.

Linearity (By HPLC)

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. In some cases, the test data may have to be subjected to a mathematical transformation before the regression analysis. Linearity was performed using five determinations covering the whole range from LOQ (0.05% of sample concentration) to 150% of the specification limit of individual limits as per ICH. Linearity was plotted by using a linear regression method to evaluate r^2.

LOD and LOQ determination (Sensitivity) (By HPLC)

LOD and LOQ of known impurities of AMD, HCTZ and OLM were performed by preparing different solutions and determining the signal-to-noise ratio. An individual analytical method's detection limit (LOD) is the lowest amount of analyte that can be detected but not necessarily quantitated as an exact value. In contrast, the quantification limit (LOQ) is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Accuracy (By HPLC)

Accuracy of the related impurities method was determined by recovery studies at four levels of concentration (LOQ, 50.0, 100.0, and 150.0%) for known impurities of AMD, HCTZ, and OLM and triplicate samples for individual concentration were injected. The recovery (%) for added known impurities of AMD, HCTZ and OLM and %relative standard deviation (RSD) was measured for individual replicate samples.

Repeatability (By HPLC)

The Repeatability (System precision and method precision) for proposed methods were performed individually by multiple measurements of standard and sample solution. A system precision was performed by five standard injections 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 (By HPLC)

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

Change in flow: ± 0.1 mL/minutes
Change in column temperature (± 5°C)
pH of the buffer: ± 0.2

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

Result and Discussion

The primary focus of the work is on general approaches and considerations toward the development and validation of chromatographic methods for related substances of AMD, HCTZ and OLM fixed-dose combination product, which can provide a versatile method in terms of cost and time to the analysts, particularly working with pharmaceuticals field to meet the current ICH and regulatory requirements. The study aimed to develop an accurate and precise stability-indicating method for related substances of the fixed-dose combination product of AML, HCTZ, and OLM. In this research work, two equivalent analytical methods are developed to separate 25 known and unknown impurities of AML, HCTZ, and OLM by HPLC and UPLC techniques within a short run time. HCTZ and its impurities are polar substances in nature, while OLM and its impurities are non-polar. To elute all the impurities of HCTZ with well retention and impurities of OLM within the shorter run time method, development was started with phenyl bonded stationary phase with a lower particle size which can give optimum retention to polar components due to polar phase and good separation due to suppression of π- π interaction. Also, lower particle size of the stationary phase increases surface area, giving more resolution between peaks within a shorter run time. Some of the impurities related to AMD and OLM are co-elution with mobile phases containing acetate and phosphate buffer at different pH (2.5 – 6.0) with organic modifier (i.e., Acetonitrile) gradient program. To optimize the mobile phase, gradient, HPLC column and diluent sequential trials were taken using various combinations of solvent and buffers. The summary of method optimization trials is given in Table 1.

Based on the development trials separation of impurities were achieved by using a mixture of 10mM Phosphate buffer pH 3.00 and Acetonitrile in the ratio of 95:5 (%v/v) as mobile phase-A and a mixture of 10mM Phosphate buffer pH 3.00 and Acetonitrile in the ratio of 10:90 (%v/v) as mobile phase-B selected for both the HPLC and UPLC method with different gradient program. A Waters X-Bridge Phenyl (4.6X150), 3.0µ column for HPLC method and Waters Acquity UPLC BEH Phenyl (2.1X75mm), 1.7µ column for UPLC method was selected as the stationary phase. The summary of method optimization trials for the UPLC method is given in Table 2.

The flow rate of 0.9 mL for HPLC and 0.3 mL for UPLC method, wavelength 237 nm, 20 µL injection volume for HPLC and 3 µL injection volume for UPLC and 30°C column temperature as the better chromatographic conditions to get better separation between all known and unknown impurities and degradation products of AMD, HCTZ and

Table 1: Method optimization summary for HPLC method

Sr. No.	*Development trial details*	*Remarks*
HPLC Method Development-Trials		
01	Column: Zorbax SB Phenyl, 4.6X250 mm, 3.5µ Column Temp: 40°C Flow rate: 1.0mL/min MP-A: 10mM Ammonium acetate pH 4.5 MP-B: Acetonitrile Diluent: Water:Methanol (30:70%v/v)	Two unknown impurities of OLM are observed at 24.7 & 25.7 min which is in tailing & fronting of AMD peak, need to modify the method to separate both the impurities from AMD peak.
02	To separate impurities column temperature was changed; Actual column temperature: 40°C Decrease temperature: 35°C Increase temperature: 45°C	The unknown peak which is detected at the tailing of AMD is separated from the main peak. But the unknown peak which is detected at the Fronting of the AMD peak is merged with AMD peak
03	To separate peak from fronting of AMD peak pH of the mobile phase was changed; Actual pH: 4.5 Decrease in pH: 4.0 Increase in pH: 5.5	OLM peak elute late than in mobile phase with pH 4.5 buffer. Both unknown peaks of OLM which was detected at the fronting & tailing of AMD is separated from the AMD peak. Unknown impurity of OLM which was detected at AMD tailing and fronting, both are separated. So, the pH 3.5 buffer needs to be used for further development. A lower particle size column with a higher carbon loan may increase the separation of peaks. So, related trails are taken in the next step.
04	In this trial, the pH of the mobile phase adjusted to 3.5 and the column changed from Zorbax SB-Phenyl to Wasters X-bridge phenyl with lower particle size (From 3.5 to 3.0µm). Column ID: Waters X-bridge Phenyl, 150×4.6mm,3.0µm	Good separation between all the peaks is observed but to get slightly more retention of HCTZ Impurity B from void pH of mobile phase to be decreased from 3.5 to phosphate buffer pH 3.0.
05	In this trial, the lower pH mobile phase was used i.e. 10 mM phosphate buffer pH 3.0 and taken trial with the same column and gradient program.	With pH 3.0 phosphate buffer observed good retention and the method is almost good in terms of separation but AMD peak is observed at the edge of the slope. Hence, the gradient to be modified.
06	With the same chromatographic conditions as per the previous trial, the gradient program was modified. Also changed mobile phase-B from Aceotnitrle (100%) to 10mM phosphate buffer:Acetonitrile (10:90%v/v).	The change in gradient peak of AMD was evaluated with appropriate retention and baseline. Small modification at post gradient is required for better saturation.
07	Modification in gradient program for fine-tuning of the method	A minor modification was done in gradient and all peaks were eluted with good separation.

Table 2. Method optimization summary for UPLC method

Sr. No.	*Development trial details*	*Remarks*
UPLC Method Development-Trials		
01	With the same chromatographic condition of optimized HPLC method used for UPLC with changing following parameters; Equivalent UPLC column (i.e. Waters Acquity UPLC BEH Phenyl (2.1X75mm), 1.7µ. The gradient program was changed accordingly. Flow rate changed with 0.3 mL/min. Injection volume changed with 3 µL.	Transformation of HPLC method to UPLC suitably ok, all peaks are observed well separated and eluted with the similarity of HPLC method elution pattern. A minor change in gradient is required for the fine-tuning of the method.
02	The gradient program was modified for fine-tuning of the method.	A minor modification was done in gradient and all peaks were eluted with good separation within 45 minutes.

OLM fixed-dose combination products. The gradient program is shown in Tables 3 and 4.

HPLC method was developed with optimum chromatographic conditions to get separation and quantification of total 28 peaks of AMD, HCTZ, OLM, and its impurities with optimum separation with symmetrical peaks. The chromatogram of the spiked sample analyzed in HPLC is shown in Fig. 7.

Same way UPLC method was developed with optimum chromatographic conditions to get separation and quantification of the total 28 peaks of AMD, HCTZ, OLM and its impurities with optimum separation with symmetrical peaks. The chromatogram of the spiked sample analyzed in HPLC is shown in Fig. 8.

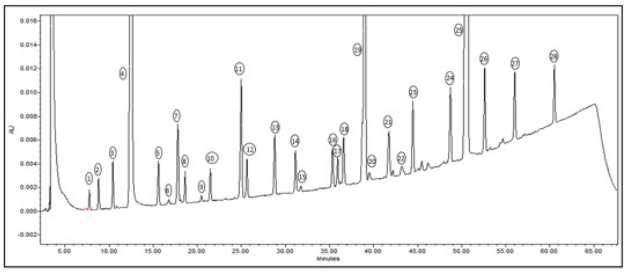
Method Validation

Specificity

Specificity was accessed by comparing chromatograms of blank, standard solution, sample solution, impurity

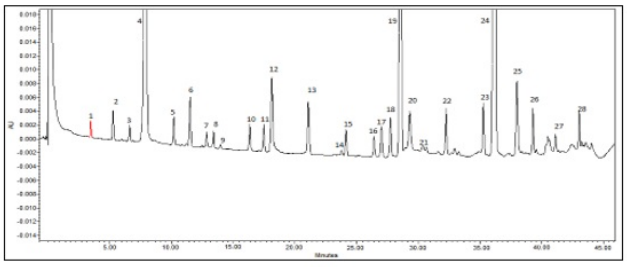
Table 3: Final gradient for HPLC method

Time (minutes)	*Mobile phase-A (%)*	*Mobile phase-B (%)*
0	90	10
3	90	10
35	60	40
55	20	80
58	40	60
62	50	50
62.1	90	10
70	90	10



1. Hydrochlorothiazide EP Impurity B
2. Hydrochlorothiazide EP Impurity A
3. 5-Chlorohydrochlorothizide
4. Hydrochlorothiazide
5. Hydrochlorothiazide EP Impurity C
6. Unknown imp of Amlodipine
7. Amlodipine EP Impurity D
8. Amlodipine EP Impurity F
9. Unknown impurity of Amlodipine
10. Amlodipine N-Lactoside impurity
11. Olmesartan EP Impurity A
12. Amlodipine Mannitol Adduct impurity
13. Amlodipine EP Impurity E
14. Amlodipine EP Impurity G
15. Unknown imp of Olmesartan
16. Amlodipine EP Impurity B
17. Amlodipine EP Impurity H
18. Amlodipine EP Impurity C
19. Amlodipine
20. Unknow Imp of Amlodipine
21. Amlodipine EP Impurity A
22. Unknown imp of Olmesartan
23. Olmesartan EP Impurity B
24. Olmesartan EP Impurity C
25. Olmesartan Medoxomil
26. Olmesartan Ethyl Ester Impurity
27. Olmesartan Methyl Ester Impurity
28. Olmesartan EP Impurity D

Fig. 7: Spiked sample chromatogram (HPLC method)



1. Hydrochlorothiazide EP Impurity B
2. Hydrochlorothiazide EP Impurity A
3. 5-Chlorohydrochlorothizide
4. Hydrochlorothiazide
5. Hydrochlorothiazide EP Impurity C
6. Amlodipine EP Impurity D
7. Unknown imp of Amlodipine
8. Amlodipine EP Impurity F
9. Unknown imp of Amlodipine
10. Amlodipine N-Lactoside impurity
11. Amlodipine Mannitol Adduct impurity
12. Olmesartan EP Impurity A
13. Amlodipine EP Impurity E
14. Unknown imp of Olmesartan
15. Amlodipine EP Impurity G
16. Amlodipine EP Impurity B
17. Amlodipine EP Impurity H
18. Amlodipine EP Impurity C
19. Amlodipine
20. Amlodipine EP Impurity A
21. Unknown imp of Amlodipine
22. Olmesartan EP Impurity B
23. Olmesartan EP Impurity C
24. Olmesartan Medoxomil
25. Olmesartan Ethyl Ester Impurity
26. Olmesartan Methyl Ester Impurity
27. Unknown imp of Olmesartan
28. Olmesartan EP Impurity D

Fig. 8: Spiked sample chromatogram (UPLC method)

spiked sample solution with all impurities (mentioned in Table1), and individual impurity solutions (HCTZ EP Impurity A, B, C, 5-Chlorohydrochlorothiazide, AMD EP Impurity A, D, Mannitol adduct, N-lactoside, OLM EP Impurity A, B, C, OLM methyl ester and ethyl ester) were

Table 4. Final gradient for UPLC method

Time (minutes)	*Mobile phase-A (%)*	*Mobile phase-B (%)*
0	95	5
2	95	5
10	75	25
25	30	70
35	10	90
35.1	95	5
45	95	5

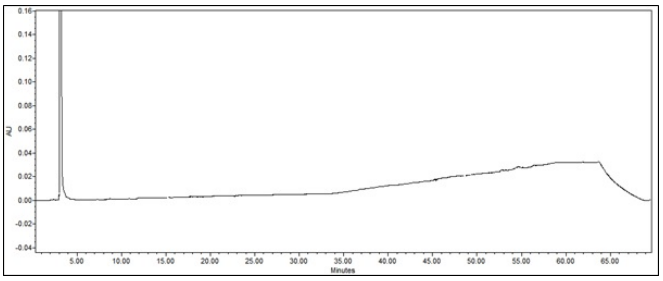


Fig. 9: Blank solution chromatogram (HPLC method)

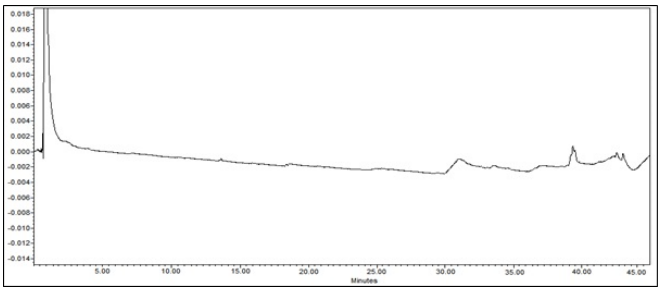


Fig. 10: Blank solution chromatogram (UPLC method)

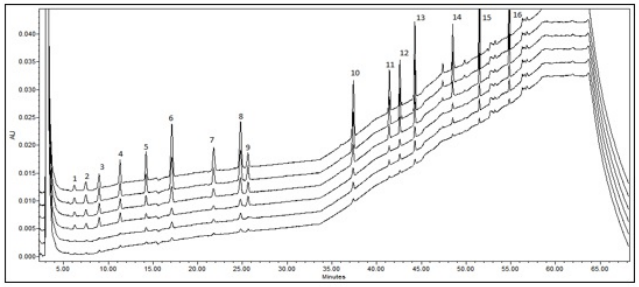
injected into HPLC and UPLC system. The chromatograms are shown in Figs. 7-10. Based on the study, there is no co-elution, and all the peaks were pure and well separated from each other. Hence, it has been proved that both the methods are specific for the intended use.

Linearity and Range

Linearity is demonstrated as the ability of the method to get a test that is directly proportional to the concentration of analyte with a defined range. The peak area was plotted against the respective concentration to get the calibration graph demonstrated in Figs. 11-14. The linearity curve shows a linear relationship over the analyte concentration range from LOQ (0.05% of sample concentration) to 150% level of specification level for HCTZ EP Impurity A, B, C, 5-Chlorohydrochlorothiazide, AMD EP Impurity A, D, Mannitol adduct, N-lactoside, OLM EP Impurity A, B, C, OLM methyl ester, OLM ethyl ester, OLM, AMD and HCTZ. Based on the regression calculation, a linear equation was obtained y=mx+c and r^2 was found greater than 0.99, representing the method is linear over the defined concentration for the intended use. Linearity values are demonstrated in Table 5.

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

The LOD is the lowest analyte level in the sample that could be detected and LOQ is the lowest analyte level in the sample that could be quantified precisely. The results presented as LOD and LOQ for impurities and API are 0.03% and 0.05% of sample concentration, respectively. The S/N ratio was determined for LOD and LOQ level concentration for each impurity and drug. S/N is observed more than 3 for LOD and more than 10 for LOQ concentration of each impurity and drug. LOD and LOQ results are summarized in Table 6.



1. Hydrochlorothiazide EP Impurity B
2. Hydrochlorothiazide EP Impurity A
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4. Hydrochlorothiazide
5. Hydrochlorothiazide EP Impurity C
6. Amlodipine EP Impurity D
7. Amlodipine N-Lactoside impurity
8. Olmesartan EP Impurity A
9. Amlodipine Mannitol Adduct impurity
10. Amlodipine
11. Amlodipine EP Impurity A
12. Olmesartan EP Impurity B
13. Olmesartan EP Impurity C
14. Olmesartan Medoxomil
15. Olmesartan Ethyl Ester Impurity
16. Olmesartan Methyl Ester Impurity

Fig. 11: Overlaid chromatograms of linearity solutions

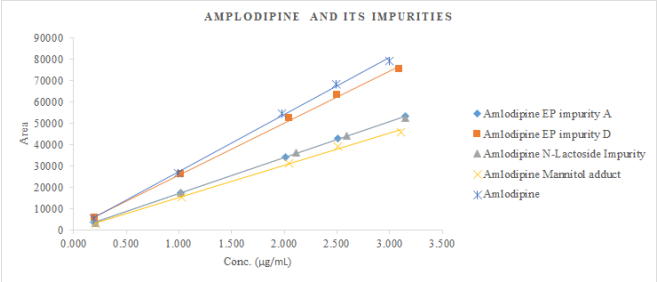

Fig. 12: Callibration curve of AMD and its impurities

Table 5: Linearity results

Component	*Slope*	*Intercept*	*Correlation coefficient*
HCTZ EP Imp A	3506.427	110.747	0.999
HCTZ EP Imp B	2287.279	121.511	0.999
HCTZ EP Imp C	10171.460	728.882	1.000
5-Chlorohydrochloro-thiazide	6485.541	234.261	0.999
HCTZ	6485.981	67.418	1.000
AMD EP Imp A	16923.804	185.891	1.000
AMDEP Imp D	24364.772	1468.626	0.999
AMD Mannitol adduct	15101.302	79.263	0.998
AMD N-Lactoside	16890.867	12.561	0.999
ALM	26792.624	398.942	0.999
OLM EP Imp A	7721.662	300.026	1.000
OLM EP Imp B	7464.896	500.388	0.999
OLM EP Imp C	8726.499	101.104	1.000
OLM Ethyl Ester	6702.991	1462.042	1.000
OLM Methyl Ester	8700.355	389.315	1.000
OLM	9073.424	1849.795	0.999

Accuracy

The accuracy of an analytical procedure describes the closeness to the accurate value generated by a method. Accuracy study was performed by spiking known impurity at different levels (LOQ, 50%, 100% and 150%) and calculated as a %recovery shown in Table 7. The accuracy result is expressed in %recovery at all four levels in the range of 90% to 110% for all impurities. The results prove that the method is accurate for the intended use.

Precision

The method's precision is derived as the closeness of agreement between a series of measurements obtained from multiple sampling of the sample from the same homogenous sample under the prescribed condition and is generally expressed as a %RSD. Based on the results of both system and method precision, the method is precise with satisfactory limits reported in Table 8.

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 the proposed method's condition. The results of robustness testing proved that the minor deliberate changes in the

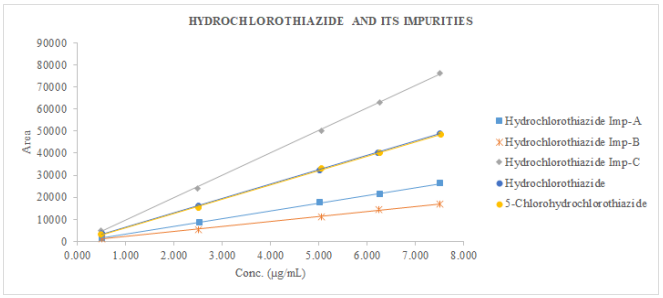

Fig. 13: Callibration curve of HCTZ and its impurities

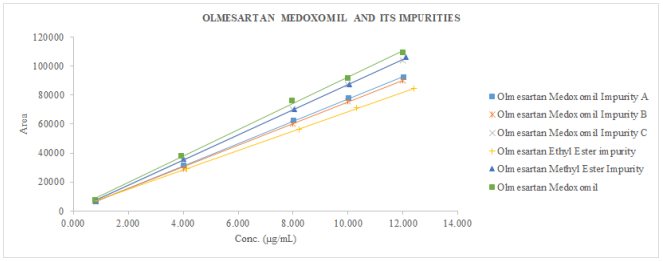

Fig. 14: Callibration curve of OLM and its impurities

Table 6: LOD and LOQ Results

	LOD		*LOQ*		
Component	*Concentration (µg/mL)*	*S/N Ratio*	*Concentration (µg/mL)*	*S/N Ratio*	*% RSD*
HCTZ EP Imp A	0.155	9	0.511	33	2.3
HCTZ EP Imp B	0.152	4	0.503	15	4.7
HCTZ EP Imp C	0.150	8	0.495	27	1.1
5-Chloro hydrochlorothiazide	0.150	7	0.495	22	2.1
HCTZ	0.152	6	0.503	15	0.9
AMD EP Imp A	0.061	5	0.201	18	1.7
AMDEP Imp D	0.060	10	0.199	39	1.3
AMD Mannitol adduct	0.063	6	0.209	24	2.9
AMD N-Lactoside	0.064	4	0.211	16	3.5
AMD	0.059	11	0.195	40	2.1
OLM EP Imp A	0.243	13	0.803	54	0.7
OLM EP Imp B	0.241	5	0.795	22	3.9
OLM EP Imp C	0.241	7	0.795	29	7.1
OLM Ethyl Ester	0.245	3	0.809	14	6.9
OLM Methyl Ester	0.245	4	0.810	19	5.5
OLM	0.239	9	0.789	838	3.4

Table 7: Accuracy Results for impurities

Impurity	*At LOQ (0.05%) level*	*At 50% level*	*At 100% level*	*At 150% level*
HCTZ EP Imp A	97.3	99.8	99.1	100.7
HCTZ EP Imp B	96.9	99.1	98.7	99.8
HCTZ EP Imp C	96.4	98.3	97.2	96.9
5-Chlorohydrochlorothiazide	97.7	98.9	100.3	98.1
AMD EP Imp A	95.2	97.3	96.9	99.2
AMD EP Imp D	97.4	98.6	97.9	100.5
AMD Mannitol adduct	98.0	99.1	98.6	98.4
AMD N-Lactoside	95.1	99.5	97.1	96.8
OLM EP Imp A	98.1	99.8	96.3	98.2
OLM EP Imp B	95.7	96.4	97.8	101.1
OLM EP Imp C	95.1	98.2	99.3	98.1
OLM Ethyl Ester	97.3	96.8	100.9	99.4
OLM Methyl Ester	96.2	99.3	98.9	100.2

Table 8: Metho precision Results for impurities

Impurity	*%Impurity*	*%RSD*
HCTZ EP Imp A	0.501	2.1
HCTZ EP Imp B	0.498	3.9
HCTZ EP Imp C	0.503	1.8
5-Chlorohydrochlorothiazide	0.509	4.1
AMD EP Imp A	0.495	4.3
AMD EP Imp D	0.502	2.6
AMD Mannitol adduct	0.496	1.1
AMD N-Lactoside	0.499	3.5
OLM EP Imp A	0.507	2.8
OLM EP Imp B	0.510	4.3
OLM EP Imp C	0.496	1.7
OLM Ethyl Ester	0.500	1.5
OLM Methyl Ester	0.501	2.3

method parameter, e.g., flow rate, column temperature, and pH of the mobile phase, are robust and within the acceptance criteria. In all modifications, system suitability was achieved, and %impurities were observed within the acceptable range. Hence, the method is robust.

Comparison of HPLC and UPLC Method

To prove equivalency between both the optimized method by HPLC and UPLC, blank, standard solution, sample solution, and spiked sample solution with all impurities were injected into both the methods. The chromatogram is given as Fig. 5. System suitability criteria, impurity elution order, and %impurity level were compared and observed similarly. Complete method validation was performed for the HPLC method. Specificity was performed for the UPLC method to check co-elution and peak purity. A comparison study proved that both the methods are equivalent and suitable for the intended use.

In the described research, a simple, accurate, precise, linear, and cost-effective analytical method by HPLC and UPLC has been developed and validated as per ICH guidelines for related impurities of AMD, HCTZ and OLM fix dose combination products. These methods are more efficient in separating many impurities by a single method. The HPLC method is capable enough to resolve 28 peaks with an optimum resolution with a short run time, while the UPLC method is superior in terms of separation of 28 peaks in almost half the run time of the HPLC method. HPLC method was validated as per ICH guidelines by proving parameters including system suitability, precision, LOD-LOQ, Linearity, accuracy, and robustness. Equivalency between both HPLC and UPLC methods was proved by performing specificity. Hence, both methods are suitable for intended use in pharmaceutical analysis to save cost and time.

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