

Stability-Indicating Method for Simultaneous Estimation of Olmesartan Medoxomile, Amlodipine Besylate and Hydrochlorothiazide by RP-HPLC in Tablet Dosage Form

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A simple, specific, accurate and precise stability-indicating reversed-phase high-performance liquid chromatographic method was developed for simultaneous estimation of olmesartan medoxomile (OLME), amlodipine besylate (AMLO) and hydrochlorothiazide (HCTZ) in tablet dosage form. The method was developed using an RP C18 base deactivated silica column (250 × 4.6 mm, 5 μm) with a mobile phase consisting of triethylamine (pH 3.0) adjusted with orthophosphoric acid (A) and acetonitrile (B), with a timed gradient program of T/%B: 0/30, 7/70, 8/30, 10/30 with a flow rate of 1.4 mL/min. Ultraviolet detection was used at 236 nm. The retention times for OLME, AMLO and HCTZ were found to be 6.72, 4.28 and 2.30, respectively. The proposed method was validated for precision, accuracy, linearity, range, robustness, ruggedness and force degradation study. The calibration curves of OLME, AMLO and HCTZ were linear over the range of 50–150, 12.5–37.5 and 31–93 μg/mL, respectively. The method was found to be sensitive. The limits of detection of OLME, AMLO and HCTZ were determined 0.19, 0.16 and 0.22 μg/mL and limits of quantification of OLME, AMLO and HCTZ were determined 0.57, 0.49 and 0.66, respectively. Forced degradation study was performed according to International Conference on Harmonization guidelines.

Introduction

Olmesartan medoxomile (OLME), chemically (5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate, is an angiotensin II receptor blocker used as an antihypertensive agent (1) (Figure 1). In the literature, several analytical methods have been reported for the determination of OLME in biological fluids and pharmaceutical formulations, including liquid chromatography coupled with mass spectrometry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC) and spectrophotometric estimation (2–5). In these methods, OLME was analyzed either alone or in combination with other drugs.

Amlodipine besylate (AMLO), chemically (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (6), is a long-acting calcium channel blocker that is used as an antihypertensive agent (7–9) (Figure 1). For AMLO, alone or in combination, several analytical methods such as HPLC and HPTLC have been reported for its estimation in biological fluids and pharmaceutical formulations (10–14).

Hydrochlorothiazide (HCTZ), chemically 6-chloro-1, 1-dioxo-3,4-dihydro-2H-1,2,4-benzothiaziazine-7-sulfonamide, is a diuretic (15) (Figure 1). Various analytical methods have been

reported for the analysis of HCTZ alone and in combination in biological fluids and pharmaceutical preparations (16–18).

A literature survey revealed that no method has been reported on these drugs in combined pharmaceutical dosage forms. Therefore, in the present study, an attempt was made to develop a simple, precise, accurate and robust and stability-indicating HPLC method on the analysis of OLME, AMLO and HCTZ in bulk and pharmaceutical formulation.

Experimental

Material and chemicals

Hydrochloric acid, sodium hydroxide and hydrogen peroxide, all reagent grade (LR grade), and acetonitrile and triethylamine (HPLC grade) were obtained from Merck Chemicals. Orthophosphoric acid (AR grade) was obtained from LOBA Chemicals. Purified water for chromatography was obtained from a Milli-Q purification unit (Millipore, Milford, MA). OLME, AMLO and HCTZ were obtained as gift samples (Torrent Pharmaceutical, Ahmadabad).

Instrumentation

High-performance liquid chromatography

An HPLC system with an Ezchrom Elite data handling system (Agilent 1200 Separation module) and a Rheodyne manual injector (20 μl) was used for the analysis. The purity determination was performed on a stainless steel column, 250 mm long, 4.6 mm internal diameter, filled with base deactivated silica chemically bonded to porous silica particles of 5 μm diameter (LCGC C18, 250 × 4.6 mm, 5 μm).

Chromatographic conditions

Mobile phase A was prepared by diluting 7 mL triethylamine to 1,000 mL with water and then adjusting its pH to 3.0 with orthophosphoric acid. Mobile phase B consisted of acetonitrile, with a timed gradient program of T/%B: 0/30, 7/70, 8/30, 10/30, in a C18 column of base deactivated silica (250 × 4.6 mm, 5 μm). The mobile phase was filtered through a 0.45-μ membrane filter and then sonicated for 10 min. The flow rate was set at 1.4 mL/min. All three drugs showed good absorbance at 236 nm, which was selected as the wavelength for further analysis, and all determinations were performed at ambient column temperature. Diluent used was mobile phase A and mobile phase B in the ratio of 60:40 v/v.

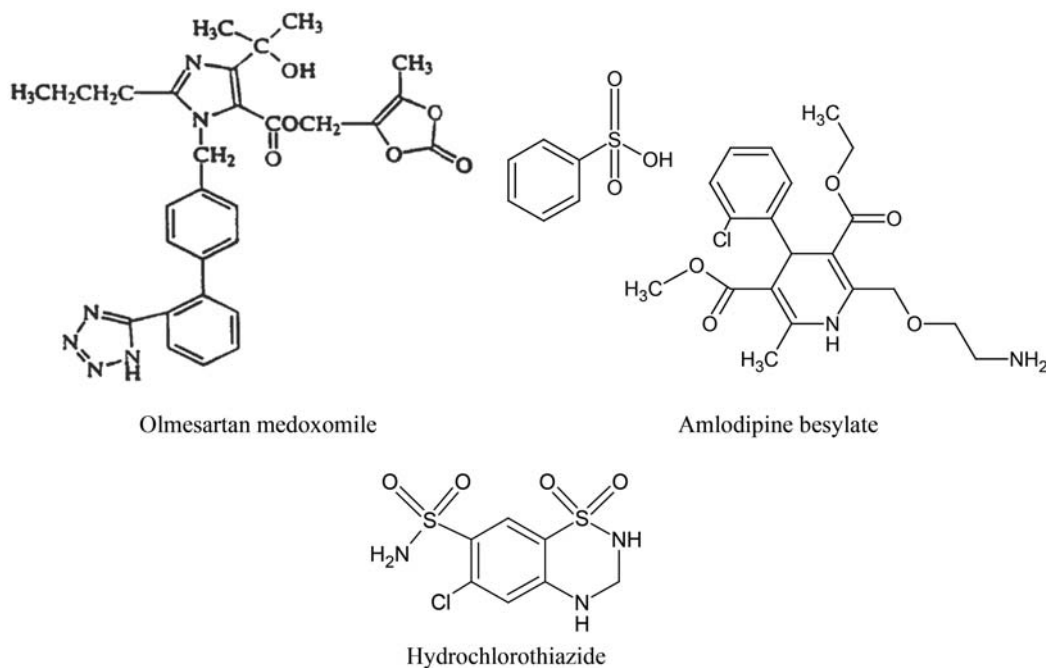


Figure 1. Structures of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide.

Preparation of stock solution and standard solution

Accurately weighed 50 mg of OLME, 25 mg of AMLO and 31 mg of HCTZ were dissolved in 50, 100 and 50-mL volumetric flasks with diluent (stock solution). The stock solution was further diluted by using the mobile phase to get concentrations of 100, 25, and 62 µg/mL of OLME, AMLO and HCTZ, respectively.

Validation of method

The developed method was validated in terms of linearity, specificity, precision, accuracy, robustness and ruggedness (19–20).

Linearity

Approximately 25 mg of OLME, 25 mg of AMLO and 31 mg of HCTZ reference standard were weighed accurately and transferred to 25, 100 and 50-mL volumetric flasks, respectively. Diluents were added, the mixture was sonicated to dissolution, then made up to the volume with diluent and mixed well. Linearity was studied by preparing standard solutions at different concentration levels.

Specificity

Blank preparation, standard preparation and placebo preparation of OLME, AMLO and HCTZ tablets (40 + 10 + 25 mg), in addition to sample preparation of OLME, AMLO and HCTZ tablets were prepared. The interference of placebo with the primary drug peak in the sample was checked using a photodiode array detector. Diluent was used as blank preparation.

Precision

For the proposed analytical procedure, the validation parameter was carried out by assay of six sample preparations under the same operating conditions (same analyst, same equipment, same day) for OLME, AMLO and HCTZ tablets.

Accuracy (recovery studies)

The recovery experiments were carried out using the following levels of the test concentration in the assay method: 80% (OLME, 80 ppm; AMLO, 20 ppm; and HCTZ, 50 ppm), 100% (OLME, 100 ppm; AMLO, 25 ppm; and HCTZ, 62.5 ppm), and 120% (OLME, 120 ppm; AMLO, 30 ppm; and HCTZ, 75 ppm).

Limit of quantitation and limit of detection

To determine limit of detection (LOD) and limit of quantification (LOQ) of OLME, AMLO and HCTZ, concentrations in the lower part of the linear range of the calibration curve were used. The LOQ and LOD were calculated using the following equation: $LOD = 3.3 \times N/B$ and $LOQ = 10 \times N/B$, where N is standard deviation of the peak areas of the drugs ($n = 3$), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

Intermediate precision (ruggedness)

Intermediate precision expresses within-laboratory variations: different days, different analysts and different equipment. Intra-day and inter-day precision of the proposed method was evaluated by assaying six freshly prepared replicates of sample solutions of OLME, AMLO and HCTZ in duplicate with concentrations of 100, 25 and 62 ppm, respectively.

Forced degradation study

Different stress conditions were used for the forced degradation studies of bulk drug and drug formulations. In this procedure, one sample made without the drug, i.e., placebo sample, and samples with drugs were compared with the forced degradation sample.

Acidic condition

For acid hydrolysis, 1N of hydrochloric acid was used for preparation of 100, 25, and 62 µg/mL of OLME, AMLO and HCTZ solution, respectively. Fifty milligrams of OLME active pharmaceutical ingredient (API), 25 mg of AMLO API and 31 mg of HCTZ API were dissolved in a 100-mL volumetric flask with 10 mL mobile phase and used to make a sample preparation for a tablet equivalent to 40 mg of OLME, 10 mg of AMLO and 25 mg of HCTZ in a 100-mL volumetric flask. Then, 5 mL of 1N hydrochloric acid were added to each flask, and the flasks were placed in a water bath at 105°C for 30 min. After this, 5 mL of 1N sodium hydroxide were added to each flask to neutralize the reaction, then the volume was made up with mobile phase. For further dilution, 5 mL of each sample was placed in a 50-mL volumetric flask individually; for tablet degradation, 5 mL were added to a 20-mL flask and made up to volume with mobile phase (Figure 3).

Alkaline condition

For base degradation, 2N sodium hydroxide was used for preparation of 100, 25, and 62 µg/mL of OLME, AMLO and HCTZ solution, respectively. OLME API (50 mg), AMLO API (25 mg) and HCTZ API (31 mg) were dissolved in 50, 100 and 50-mL volumetric flasks with 10 mL of mobile phase, respectively, and sample preparations were made for tablets equivalent to 40 mg of OLME, 10 mg of AMLO and 25 mg of HCTZ in a 100-mL volumetric flask. Then, 10 mL of 2N sodium hydroxide were added to each flask, and the flasks were placed under a water bath for 90 min at 105°C. After this, 10 mL of 2N hydrochloric acid were added to each flask to neutralize the reaction, then the volume was made up with mobile phase. For further dilution, 5 mL of each sample was added individually to a 50-mL volumetric flask, and for tablet degradation, 5 mL were added to a 20-mL flask and the volume was made up with mobile phase (Figure 4).

Oxidation condition

For peroxide degradation, 10% hydrogen peroxide was used for preparation of 100, 25, and 62 µg/mL of OLME, AMLO and HCTZ solutions, respectively. OLME API (50 mg), AMLO API (25 mg) and HCTZ API (31 mg) were dissolved in 50, 100 and 50-mL volumetric flasks with 10 mL mobile phase, and sample preparations were made for tablets equivalent to 40 mg of OLME, 10 mg of AMLO and 25 mg of HCTZ in a 100-mL volumetric flask. Five milliliters of 10% hydrogen peroxide were added to each flask and exposed to a 105°C water bath for 45 min, then made up with mobile phase. For further dilution, 5 mL of each sample were placed individually in a 50-mL volumetric flask, and for tablet degradation, 5 mL were placed in a 20-mL flask and the volume was made up with mobile phase (Figure 5).

Thermal degradation

For thermal degradation, 10 mg of the drugs were kept in a hot air oven for 48 h at 1,00°C, then made up with mobile phase. For further dilution, 5 mL of each sample were placed individually in a 50-mL volumetric flask, and for tablet degradation, 5 mL were placed into a 20-mL flask and made up with mobile phase.

Photo degradation

For photo degradation, 10 mg of the drugs were exposed to a short wavelength (254 nm) and a long wavelength (366 nm) of ultraviolet (UV) light for 48 h, then made up with mobile phase. For further dilution, 5 mL of each sample were placed individually in a 50-mL volumetric flask, and for tablet degradation, 5 mL were placed in a 20-mL flask and made up with mobile phase.

Assay preparation of OLME, AMLO and HCTZ in tablet dosage forms

For the assay, a strength of 40 + 10 + 25 mg of OLME, AMLO and HCTZ was used. The 20 tablets were taken, accurately weighed and finely powdered. All crushed powder was placed into a 200-mL volumetric flask and dissolved in 150 mL of mobile phase, ultrasonicated for 25 min and filtered through Whatman filter paper. The volume was made up to 200 mL and from this, 5 mL was taken and diluted to 200 mL in a volumetric flask. Final concentrations of OLME, AMLO and HCTZ were 100, 25 and 62.5 µg/mL, respectively. The diluted solution was analyzed under optimized chromatographic conditions. (Figure 2B).

Results and Discussion

Optimization of chromatographic conditions

The primary target in developing this stability-indicating HPLC method is to achieve resolution between all three drugs and its degradation products. To achieve the separation of degradation products, we used a stationary phase C-18. A mobile phases consisting of different buffers with methanol and acetonitrile at different buffer–methanol and acetonitrile ratios and at different mobile phase pH were tried, but peak shapes and retention times of OLME, AMLO and HCTZ were broad compared to buffer–acetonitrile composition as mobile phase. After various trials of different buffer and acetonitrile ratios as mobile phase, potassium dihydrogen phosphate was selected as buffer, pH was adjusted to 3.0 with orthophosphoric acid and buffer–acetonitrile ratio was selected at a proportion of 70:30. This showed a good resolution chromatogram with symmetrical peaks (Figure 2A). The calibration graphs for OLME, AMLO and HCTZ were constructed by plotting the peak area versus their corresponding concentrations, respectively; good linearity for both was found over the range of 50 to 150 ppm for OLME, 12.5–37.5 ppm for AMLO and 31–93 ppm for HCTZ. To develop a simple, specific, accurate and precise RP-HPLC method for simultaneous estimation of OLME, AMLO and HCTZ, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. System suitability tests were carried as per International Conference on Harmonization (ICH) guidelines and parameters are summarized in Table I.

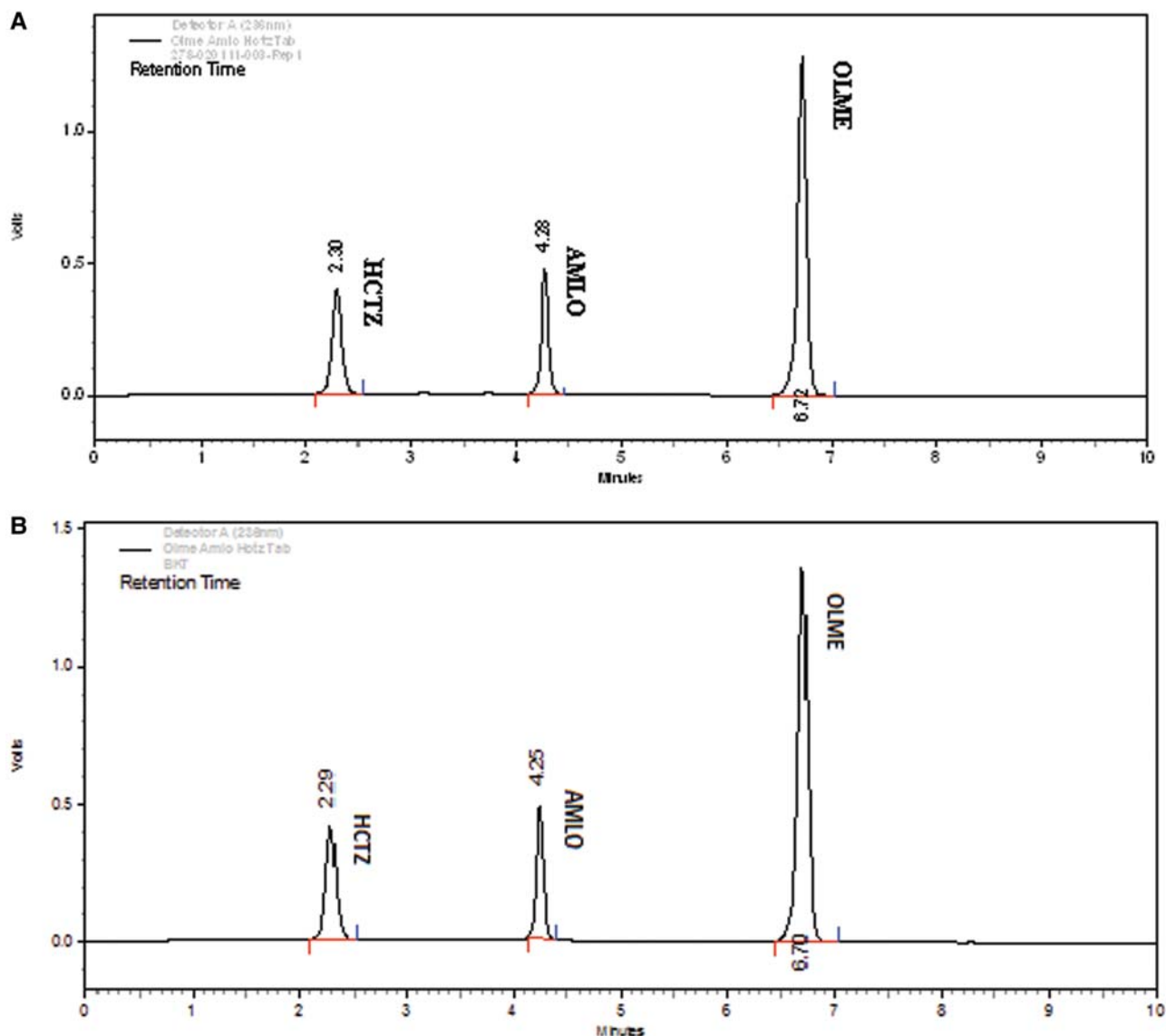


Figure 2. HPLC chromatograms of olmesartan, amlodipine and hydrochlorothiazide: standard (A); sample (B).

Table I
Results of System Suitability Parameters of the Developed Method for Determination of OLME, AMLO and HCTZ*

Compound	Retention time	%RSD of retention time	%RSD of area	Asymmetry	Theoretical Plates
OLME	6.72	0.13	0.16	1.43	9,289
AMLO	4.28	0.20	0.90	1.23	5,973
HCTZ	2.30	0.53	0.16	1.18	2,932

*Average of five readings.

Method validation

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity ranges for OLME, AMLO and HCTZ were found to be 50–150, 12.5–37.5 and 31–93 µg/mL, respectively. The regression equations for OLME,

AMLO and HCTZ were found to be $y = 74865x - 48536$, $y = 93447x - 15218$ and $y = 34024 + 18794$ with correlation coefficients (R^2) 0.999, 0.999 and 0.999, respectively.

Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants or matrix. Specificity of an analytical method is its ability to accurately and specifically measure the analyte of interest without interference from blank or placebo. The peak purities of OLME, AMLO and HCTZ were assessed by comparing the retention times of standard OLME, AMLO and HCTZ and the sample, and good correlation was obtained between the retention time of the standard and sample. Placebo and blank were injected and there were no peaks. No interference was found; hence, the method is specific.

Precision

Precision was evaluated by carrying out six independent sample preparations of a single lot of formulation. The sample preparation was carried out in same manner as described previously. The percent assay was found to be 98.6–99.8, 98.1–99.9, 96.0–98.2% of OLME, AMLO and HCTZ, respectively. The percentage relative standard deviation (%RSD) was found to be less than 2%, which that proves that the method is precise, as shown in Table II.

Accuracy (recovery studies)

To check the accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80, 100 and 120% concentration levels. Known amounts of standard OLME, AMLO and HCTZ were added to the pre-analyzed samples and subjected to the proposed HPLC method. The percent recovery was found to be within the limits of the acceptance criteria with average recoveries of 99.3–100.03% for OLME, 98.9–100.1% for AMLO and 97.53–98.75% for HCTZ. Results of recovery studies are shown in Table III.

Limit of quantitation and limit of detection

Limit of quantitation (LOQ) and limit of detection (LOD) can be determined based on visual evaluation, signal-to-noise approach and standard deviation of the response and slope. The LOD of OLME, AMLO and HCTZ was determined 0.19, 0.16 and 0.22, respectively. The LOQ of OLME, AMLO and HCTZ was determined 0.57, 0.49 and 0.66, respectively.

Table II
Precision Studies*

Drug	Precision % ± SD	%RSD	Intermediate precision	%RSD
OLME	99.3 ± 0.68	0.40	98.9 ± 0.48	0.68
AMLO	98.6 ± 0.59	0.53	99.9 ± 0.39	0.60
HCTZ	98.6 ± 0.70	0.87	98.7 ± 0.56	0.71

*Average of 6 readings.

Table III
Recovery Studies of OLME, AMLO and HCTZ (n = 6)

Excess drug added to the analyte (%)	OLME recovery (%)	Mean recovery (%)	Mean %RSD
80	99.3		
100	99.7	99.67	0.13
120	100.03		
Excess drug added to the analyte (%)	AMLO recovery (%)	Mean recovery (%)	Mean %RSD
80	98.9		
100	99.81	99.60	0.13
120	100.1		
Excess drug added to the analyte (%)	HCTZ recovery (%)	Mean recovery (%)	Mean %RSD
80	97.53		
100	98.75	98.31	0.33
120	98.31		

Robustness of the method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were made. The effect of change in flow rate and change in pH retention time, tailing factor and theoretical plates were studied. The method was found to be unaffected by small changes like ±10% in flow rate or ±0.2 change in pH, as shown in Table IV.

Intermediate precision (ruggedness)

Different analysts carried out precision studies in a similar manner as that used by the first analyst. The percent assay was found to be 98.2–100.3, 97.4–99.1 and 97.0–98.6% of OLME, AMLO and HCTZ, respectively. %RSD was found to be less than 2%, which proves that the method is rugged, as shown in Table II.

Results of force degradation study

Degradation was not observed for OLME, AMLO and HCTZ samples during stress conditions like heat, UV and light: they show only one peak of drug under these stress conditions. However, degradation was observed in base, acid and oxidation. OLME, AMLO and HCTZ samples were degraded into acid (Figure 3), base (Figure 4) and oxidation (Figure 5) and formed polar impurities. Greater peak purity results indicate that the OLME, AMLO and HCTZ sample peaks are homogeneous under all tested stress conditions. The unaffected assay of samples in the tablet confirms the stability-indicating power of the method.

The percent recovery of acid degradation in API was found to be 66.82, 61.27 and 85.71 of OLME, AMLO and HCTZ, respectively. The percent recovery of acid degradation in the tablet was found to be 71.49, 64.32 and 80.43% of OLME, AMLO and HCTZ, respectively. The percent recovery of base degradation in API was found to be 18.05, 70.27 and 67.2 of OLME, AMLO and HCTZ, respectively. The percent recovery of base degradation in the tablet was found to be 21.94, 78.31 and 71.51 of OLME, AMLO and HCTZ, respectively. The percent recovery of peroxide degradation in API was found to be 32.11, 25.60 and 63.76 of OLME, AMLO and HCTZ, respectively. The percent recovery of peroxide degradation in the tablet was found to be 41.19, 31.58 and 78.41 of OLME, AMLO and HCTZ, respectively (Table V).

The results of the stress studies indicated the specificity of the developed method. OLME degraded more in base than in acid and oxidative stress conditions, whereas AMLO degraded more in oxidative than other conditions. HCTZ was found to be more stable than OLME and AMLO in all three conditions.

Table IV
Results of Robustness Study

Parameter	Retention time			Asymmetry			Theoretical plates			
	OLME	AMLO	HCTZ	OLME	AMLO	HCTZ	OLME	AMLO	HCTZ	
pH of mobile phase	2.8	7.14	4.33	2.32	1.39	1.21	1.19	8,904	5,917	2,385
Flow rate(mL/min)	1.3	7.37	4.35	2.38	1.45	1.26	1.21	9,871	5,846	3,092
	1.5	5.99	3.55	2.08	1.43	1.24	1.17	8,734	5,138	1,936

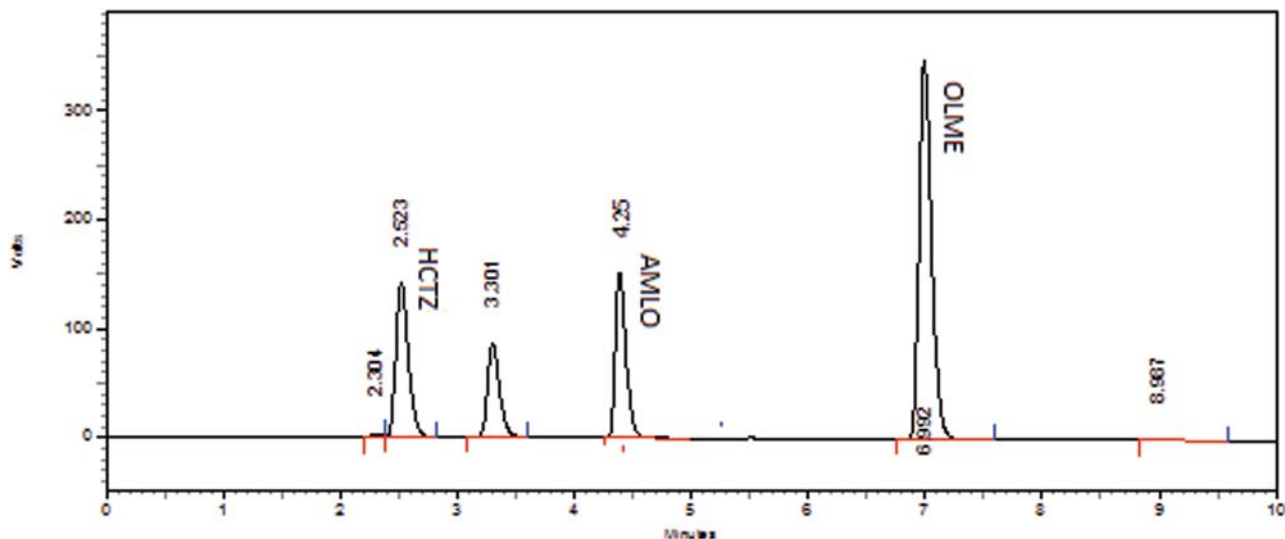


Figure 3. HPLC chromatograms of acid degradation of tablet.

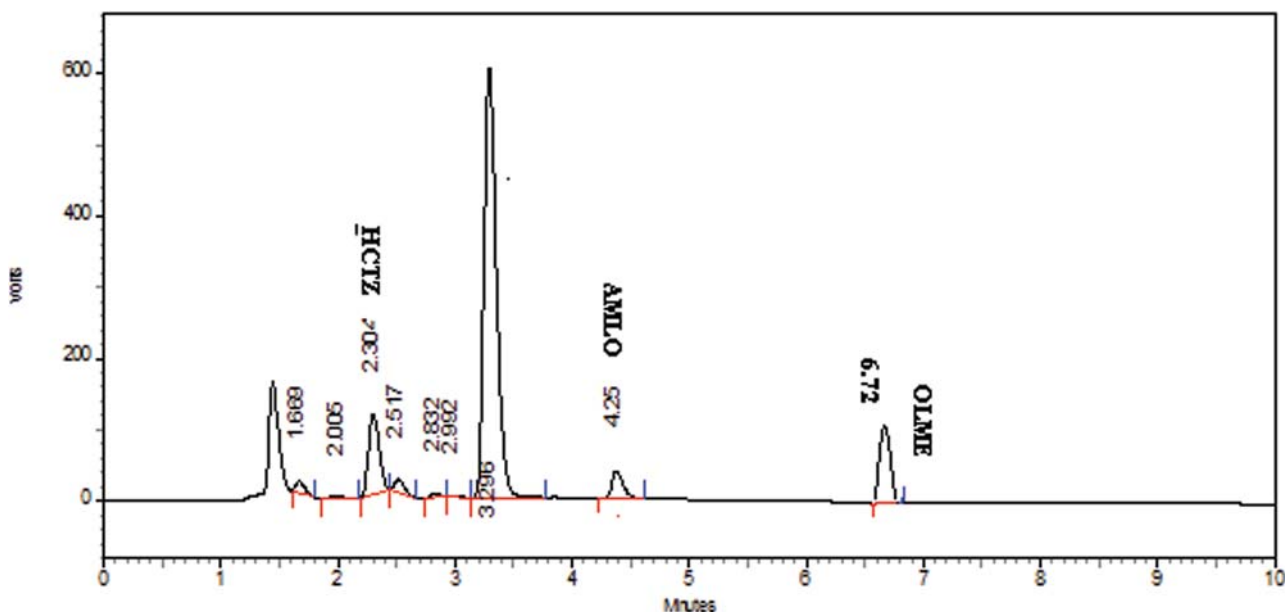


Figure 4. HPLC chromatograms of base degradation of OLME, AMLO, HCTZ and tablet.

These drugs were found to be more stable under acidic conditions than under alkali stress conditions.

Assay results of tablet dosage forms

The percent assay was found to be 99.33 ± 0.25 , 99.07 ± 0.15 and 98.50 ± 0.36 of OLME, AMLO and HCTZ in tablet dosage forms.

Conclusion

The present study was conducted to develop and validate a simple, sensitive and reproducible stability-indicating RP-HPLC

method for quantitative determination of OLME, AMLO and HCTZ. OLME and AMLO were found to be unstable under alkaline and peroxide degradation, and were less stable under acidic conditions. HCTZ was found to be unstable under peroxide conditions, and was less stable under acidic and alkaline conditions. The developed chromatographic assay fulfilled all the requirements to be identified as simple, specific, selective and reliable, including accuracy, linearity, recovery and precision data.

The data generated from the forced degradation studies enabled the evaluation of OLME, AMLO and HCTZ stability under a variety of ICH recommended conditions. These data are valuable for the safety and potency assessment of a drug product. Furthermore, this simple and rapid RP-HPLC method

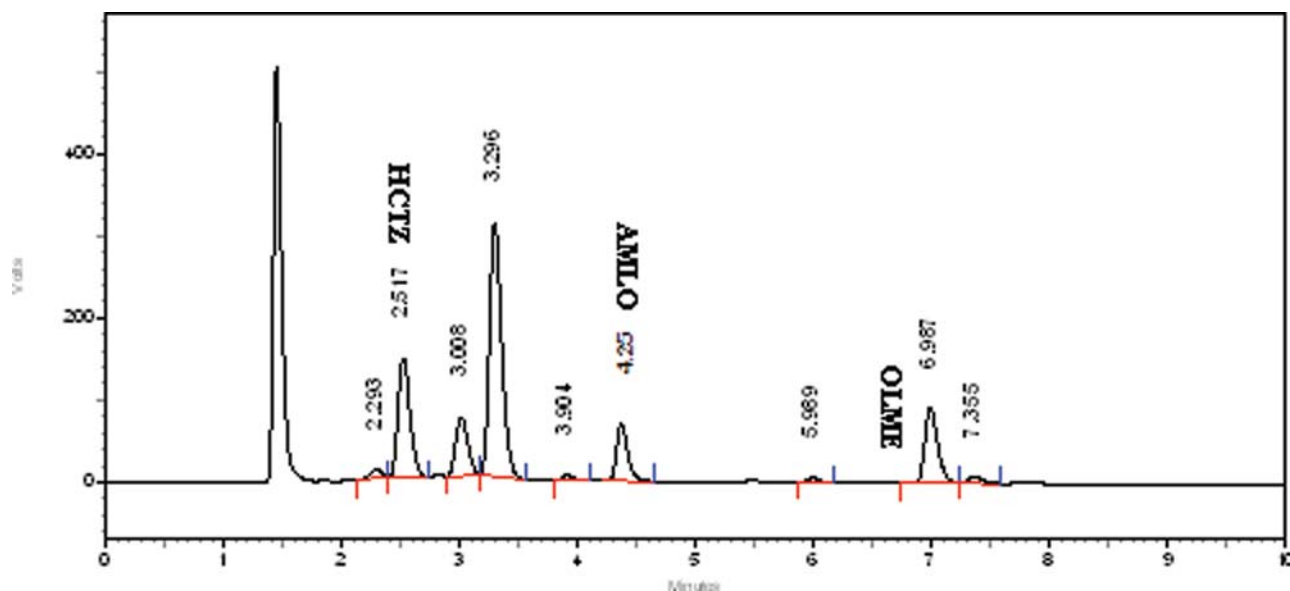


Figure 5. HPLC Chromatograms of peroxide degradation of OLME, AMLO and HCTZ tablet.

Table V
Force Degradation Studies of OLME, AMLO and HCTZ

Degradation type	% OLME API recovery	% AMLO API recovery	% HCTZ API recovery	Sample degradation		
				% OLME recovery	% AMLO recovery	% HCTZ recovery
Acid	66.82 ± 0.12	61.27 ± 0.31	85.71 ± 0.24	71.49 ± 0.42	64.32 ± 0.43	80.43 ± 0.24
Base	18.05 ± 0.21	70.27 ± 0.13	67.2 ± 0.27	21.94 ± 0.19	78.31 ± 0.32	71.51 ± 0.34
Oxidation	32.11 ± 0.17	25.60 ± 0.22	63.76 ± 0.31	41.19 ± 0.51	32.58 ± 0.28	78.41 ± 0.49

can also be used successfully for the determination of OLME, AMLO and HCTZ in pharmaceutical formulations without any interference from the excipients. Therefore, the proposed chromatographic procedure is confirmed as a stability-indicating method.

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