FORCED DEGRADATION STUDIES OF OLMESARTAN MEDOXOMIL AND CHLORTHALIDONE: DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method was developed for the simultaneous determination of olmesartan medoxomil (OLME) and chlorthalidone (CHLOR) in tablet dosage form. The analysis was performed on Inertsil ODS C18 (250 x 4.6 mm, 5 µ) using KH_2PO_4 phosphate buffer (pH) and acetonitrile as mobile phase in the proportion of 60: 40 V/V at flow rate of 1.0 mL/min. Detection of drugs was carried out in isocratic mode using UV detector at 275 nm. The retention time of OLME and CHLOR was 13.9 ± 0.1 min. and 4.4 ± 0.5 min., respectively and the total run time was 20 min. The method was validated according to the requirements of the United States Pharmacopeia. The percentage recoveries was found to be in the range of 98.9 - 100.7%. The method was successfully applied to the assay of OLME and CHLOR in tablet dosage form.

Keywords: Olmesartan medoxomil, chlorthalidone, simultaneous estimation, stability indicating assay, RP-HPLC, chromatography

INTRODUCTION

Forced degradation of pharmaceutical dosage forms is a vital criterion for the product quality control and marketing approval. It is used to evaluate the stability of formulations at stress conditions. Thus, development of a simple and accurate stability indicating assay method is desirable[1].

Olmesartan medoxomil (OLME) and chlorthalidone (CHLOR) are approved as a new fixed-dose combination for treatment of hypertension. OLME is described chemically as the (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[20-(1*H*-tetrazol-5-yl)[1,10-biphenyl]-4-yl]methyl}-1*H*-imidazole-5-carboxylic acid. It is a pro-drug and is hydrolyzed to olmesartan during absorption from the gastrointestinal tract. OMLE is a selective AT1 subtype angiotensin II receptor antagonist[2-5]. CHLOR is a white crystalline powder which is practically insoluble in water and freely soluble in methanol. Chemically, it is 2-chloro-5-[(1*RS*)-1-hydroxy-3-oxo-2, 3-dihydro- 1*H* isoindol-1-yl] benzenesulphonamide[6]. CHLOR is a diuretic drug which increases the rate of urine flow. The molecular structures of OLME and CHLOR are depicted in Fig. 1.

Various analytical methods have been reported for analysis of OLME and CHLOR alone or in combination with other drugs in pharmaceutical formulations and/or biological fluids, including LC-MS-MS[7,8], HPTLC[9,10], spectrophotometry[11] and HPLC[12].

In this study, a simple stability indicating RP-HPLC method was developed for the simultaneous determination of OLME and CHLOR in tablet dosage form. This method would help to quantify the drug in dosage form and in the presence of stress degradation products. The method was validated according to the requirements of United States Pharmacopeia (USP)[13] and International Council for Harmonization (ICH) guidelines[14].

MATERIALS AND METHODS:

CHLOR (purity, 100.0% w/w) and OLME (purity 99.72% w/w) were provided as gift samples from Ipca Laboratories, Mumbai, India and Zydus Pharma Ltd, Gujarat, India, respectively. All solvents were of HPLC/ AR grade. Acetonitrile (HPLC grade), *ortho*-phosphoric acid (OPA) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from SD Fine Chemicals Ltd., Mumbai, India. Milli-Q water filter through Millipore system was used thoughout the study for preparation of chemicals. Marketed formulations of fixed-dose combination OLME and CHLOR were purchased from the local market.

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Table I: Results of system suitability parameters

Parameters	OLME*	CHLOR*	Required limits
Peak area	1592685 ± 1.39	324930.3 ± 1.17	--
Retention time (min)	16.2 ± 0.03	4.4 ± 0.01	--
Tailing factor (T)	1.38 ± 0.042	1.32 ± 0.024	T < 2
Theoretical plates (N)	4333 ± 1.25	4061 ± 1.43	N > 2000
Resolution (R)	16.65 ± 1.58		R >2
** Results are mean of three determinations (n=3) ± RSD*			

Table II: Results of linearity study

Parameters	OLME	CHLOR
Linearity Range (µg/mL)	10-200 µg/mL	10-200 µg/mL
Slope	42263	12222
Intercept	50377	19839
Correlation Cofficient r2	0.999	0.999
LOQ (µg/mL)	1.717	0.125
LOD (µg/mL)	0.566	0.041
Regression equation	y = 42263x + 50377	y = 12222x + 19839
** Results are mean of three determinations n=3*		

Table III: Results of recovery study

	Level	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	%RSD
OLME	80%	32	31.91	99.97	0.671
	120%	48	48.47	100.97	0.429
CHLOR	80%	20	20.34	101.7	0.4298
	120%	30	29.98	99.93	1.1779
** Results are mean of three determinations n=3*					

Table IV: Results of precision study

Drug	Intraday Precision (% RSD)	Interday Precision (% RSD)
OLME	0.4378	0.2904
CHLOR	0.0466	0.8563
** Results are mean of three determinations n=3*		

The analysis of the drug was carried out on Agilent (1260 Infinity Series) HPLC equipped with photodiode array (PDA) detector, quaternary pump and 100 µl variable auto-sampler and OpenLab software. Chromatographic analysis was performed using Intertsil C-18 column (250 x 4.6 mm i.d., 5µm particle size). Isocratic elution with acetonitrile: buffer 40: 60 (V/V) was selected with a flow rate of 1 mL/ min. The detection wavelength was set at 275 nm with a runtime of 20 min. A double beam spectrophotometer (Shimadzu UV 1800) with 1 cm matched quartz cell was used for selection of wavelength of chromatographic detection.

Preparation of drug solution and mobile phase

Stock solutions were prepared by dissolving drug equivalent to 50.0 mg of OLME and CHLOR separately in 30 mL of acetonitrile in 50 mL volumetric flasks. The flask was sonicated for 2 min and volume was made up to the mark with acetonitrile to give a solution containing 1000 µg/mL drug solution. Aliquots of these stock solutions were diluted with mobile phase to get reference test concentrations of 40 and 25 µg/mL of OLME and CHLOR, respectively.

Sample solution of tablet dosage form was prepared by dissolving quantity of tablet powder (20 tablets) equivalent to 50 mg OLME and CHLOR in acetonitrile. This stock

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Captions missing

Table V: Results of robustness study

Parameters	% RSD	
	OLME	CHLOR
pH 2.99	1.685	1.302
pH 3.1	0.657	1.332
Flow rate 0.9	0.316	1.194
Flow rate 1.1	0.384	1.455
Mobile phase concentration 38:62	0.288	1.237
Mobile phase concentration 42:58	0.414	1.419
** Results are mean of three determinations n=3*		

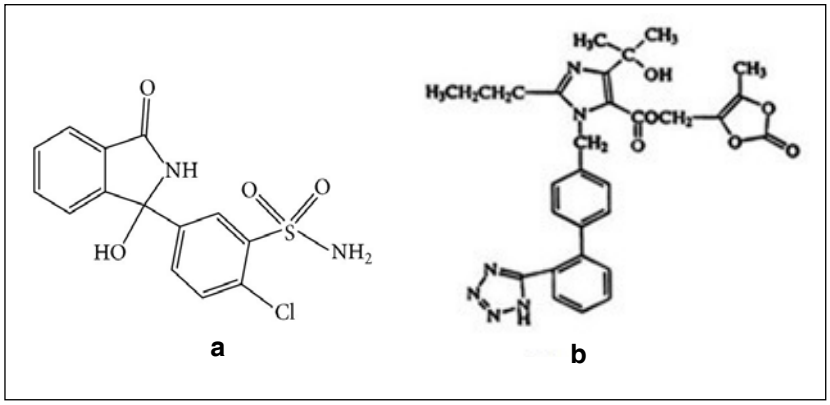

Fig. 1: Chemical structure of (a) CHLOR and (b) OLME

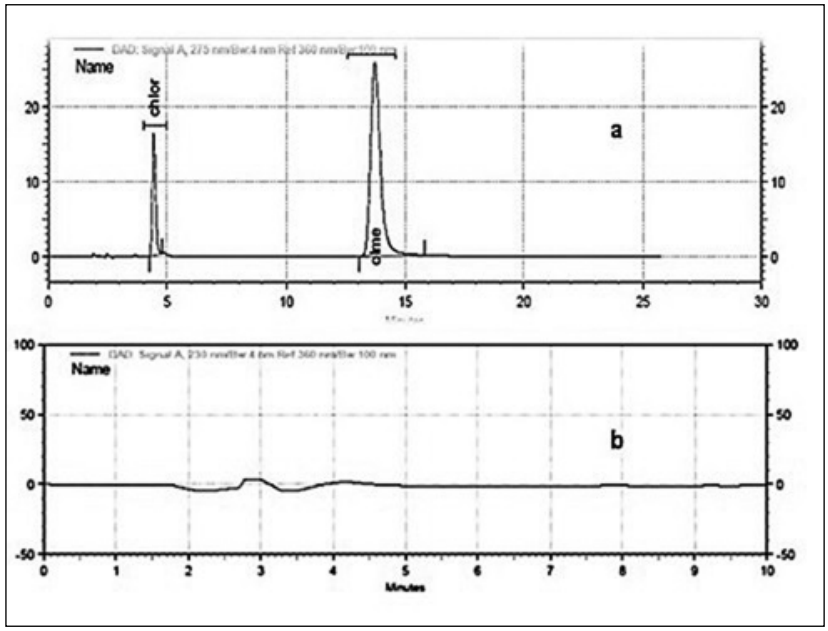

Fig. 2: Optimized chromatogram of (a) composite sample of OLME and CHLOR and (b) placebo

solution was filtered through 0.2µ filter. Aliquot (10 mL) of this stock solution was further diluted with mobile phase to 100 mL.

Potassium dihydrogen orthophosphate (KH_2PO_4) 50 mM solution was prepared by dissolving 6.83 g in about 1.0 litre purified water. The pH of this solution was adjusted to 3.0. Mobile phase was prepared by mixing phosphate buffer (pH 3.0) and acetonitrile in the ratio 60:40 V/V and filtering through a 0.2 µ nylon membrane filter.

Method Development and Optimization

The UV absorption spectrum of mixture containing 10 µg/mL each of CHLOR and OLME was recorded on spectrophotometer in the range of 400-200 nm for determination of wavelength of detection. 275 nm was selected as the most suitable wavelength for estimation of both drugs. The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the OLME and CHLOR. The acceptable results were obtained with mobile phase containing acetonitrile and phosphate buffer (pH 3.0) 60: 40 V/V.

Reference solutions of OLME and CHLOR individually and mixture were analyzed by isocratic elution of mobile phase with different ratios of phosphate and organic modifiers (acetonitrile and methanol) over different stationary phases columns at various flow rates for method optimization. Combination of acetonitrile and phosphate buffer (pH 3.0) 60: 40 V/V at a flow rate of 1.0 mL/min using Intertsil C-18 column (250 x 4.6 mm i.d., 5µm particle size) gave better resolution and symmetrical peaks for both analytes. The typical chromatograms of OLME and CHLOR in optimized chromatographic conditions are shown in Fig. 2. The optimized set of chromatographic conditions was further validated for as per USP and ICH guidelines.

METHOD VALIDATION

The analytical method was properly validated according to the requirements of USP[13] and ICH Q2 (B) guidelines[14] for accuracy, precision, intermediate precision and linearity. Specificity, limit of detection (LOD), and limit of quantification (LOQ) were established and evaluated.

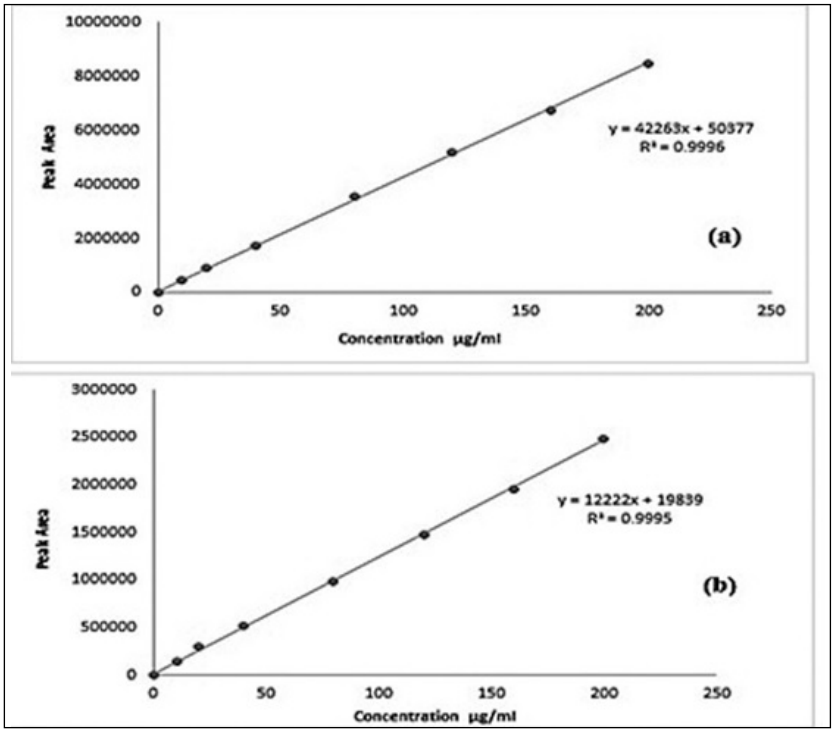

Fig. 3: Linearity of (a) OLME and (b) CHLOR

Table VI: Results of solution stability study

	% Estimation	
	OLME	**CHLOR**
24 h	100.26 ± 0.31	99.51 ± 0.27
48 h	99.42 ± 0.40	100.08 ± 0.52
72 h	99.17 ± 0.26	98.49 ± 0.40

** Results are mean of three determinations n=3 ± RSD*

System suitability was carried out with six injections (n=6) of 40 µg mL^{-1} of OLME and 25 µg/mL of CHLOR into the chromatographic system. The theoretical plates (N), tailing factor (T) and resolution were calculated. Results are shown in Table I.

For linearity study, concentrations range of 10-200 µg/mL of OLME and 10-200 µg/mL of CHLOR were analyzed. Appropriate aliquots were pipetted out from working stock solution to obtain series of concentrations. Each solution was injected in triplicate (n=3). Calibration curves were plotted with observed peak areas against concentration and regression equations and correlation coefficients was observed.

The accuracy of the analytical procedure was established by calculating percentage recovery of OLME and CHLOR. For both the drugs, recovery studies were carried out by adding known amount of OLME and CHLOR corresponding to 80, 100 and 120% of test concentration to the sample solution (n=3). The % recovery and relative standard deviation (RSD %) was estimated.

Precision study was performed by repeatability and intermediate precision study. The repeatability study was carried out recording chromatograms of six replicates of OLME and CHLOR and determining the relative standard deviation (RSD %). The intermediate precision study was carried out as intraday and interday study by estimating the corresponding responses 3 times on the same day (intraday) and on 3 different days (interday) for the test concentrations of OLME and CHLOR, respectively. The overall RSD % for peak responses in repeatability and intermediated precision study were obtained.

Robustness of the method was checked by making small but deliberate changes from the original conditions like mobile phase composition, flow rate and pH of mobile phase. The impact of these variations was determined by evaluating the value of RSD % of peak response and retention times and compared with acceptable limits (±2 %).

Specificity of the method is important to check interference of excipients on the response of the drug substance. A placebo solution was prepared from excipients of tablets in the selected mobile phase and it was analyzed using the optimized chromatographic conditions. The reference solutions of OLME and CHLOR were spiked in placebo solution separately and chromatographed to confirm the placebo interference.

Stability of sample solutions was assessed by analyzing three concentrations 20, 40, 60 µg mL^{-1} of OLME and 12.5, 25, 37.5 µg/mL of CHLOR. Replicates (n = 3) were analyzed for 72h at ambient temperature. Results were evaluated by comparing with assay results of freshly prepared solutions of reference standards. LOD and LOQ were calculated using the expression 3.3 δ/slope and 10 δ/slope, respectively.

ASSAY OF TABLET DOSAGE FORM

Twenty tablets were weighed and powdered. The quantity of OLME equivalent to 50 mg was weighed and transferred to a 50 mL volumetric flask. A volume of 25

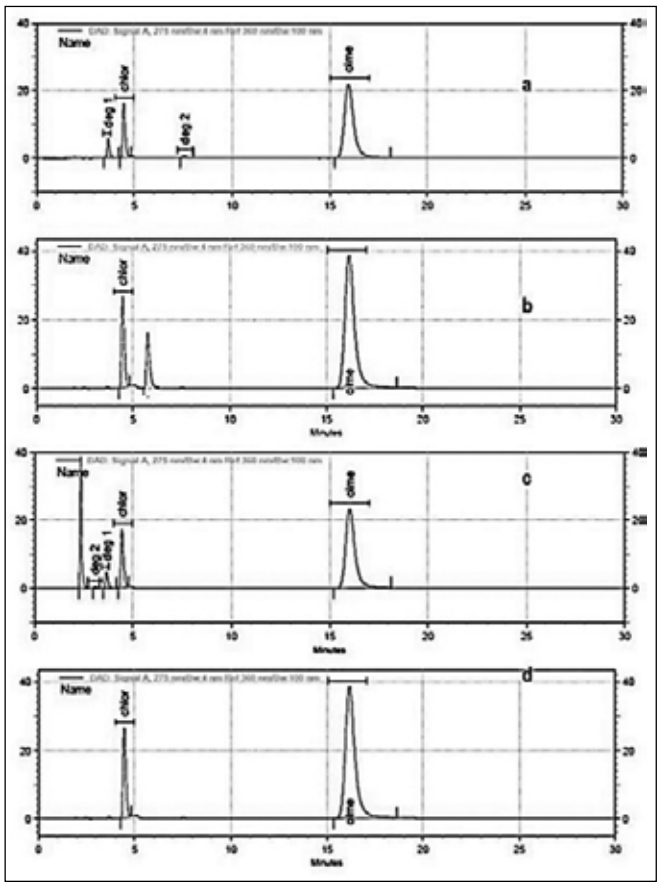

Fig. 4: Chromatograms of forced degradation study of (a) acidic, (b) alkaline, (c) oxidation, and (d) thermal degradation conditions

mL of acetonitrile was added and it was sonicated for 15 min. The volume was made us with solvent. The stock solution was diluted to obtain test concentrations of 40 µg/mL of OLME and 25 µg/mL of CHLOR. The samples (n= 3) were injected to chromatographic system and the peak responses were measured. The % content of drugs in formulation was calculated.

FORCED DEGRADATION STUDY

A volume of about 1 mL from 400 µg/mL and 250 µg/mL stock solution of OLME and CHLOR, respectively, was transferred to 10 mL volumetric flask to which 1mL 3% H_2O_2 solution was added. After 24 h, volume was made up with mobile phase and diluted to get 40 µg/mL of OLME and 25 µg/mL of CHLOR. This solution was injected in HPLC system and the percentage of degradation was calculated.

A volume of about 1 mL from 400 µg/mL and 250 µg/mL stock solution of OLME and CHLOR, respectively, was transferred to 10 mL volumetric flask. To this 2mL 0.01 N HCl and 0.01 N NaOH was added separately. The solutions were neutralized and aliquots (1mL) were diluted to 10 mL with mobile phase after 24h of exposure. The samples were injected in HPLC system to determine the degradation of drugs.

The measured amounts of CHLOR and OLME were spread on petri plates and placed in hot air oven at 120°C for 24 h. After exposure to light, weighed quantities of CHLOR and OLME were transferred in 10 mL volumetric flask and dissolved in acetonitrile. Aliquot (1mL) of each drug stock solution was diluted with mobile phase to get 40 and 25 µg/mL of PLME and CHLOR, respectively. This solution was injected in HPLC system and peak area of chromatograms of each drug was recorded to determine degradation of drugs.

RESULTS AND DISCUSSION

A combination of acetonitrile and phosphate buffer (pH 3.0) 60: 40 V/V at a flow rate of 1.0 mL min^{-1} using Intertsil C-18 column (250 x 4.6 mm i.d., 5µm particle size) was selected as optimized chromatographic condition and system suitability was assessed according to USP guidelines. The optimized chromatogram of OLME and CHLOR is depicted in Fig. 2.

Different chromatographic parameters like peak area (A), retention time (Rt), theoretical plates (N), symmetry factor (As), and resolution of OLME and CHLOR were calculated for peak response using OpenLab software of instrument. The results (Table I) showed that all the performance parameters of the analytical method comply with USP specifications for system suitability. The RSD for peak area and retention time of both analytes was less than 2.0%, resolution was more than 2.0, tailing factor was less than 2.0, and the number of theoretical plates were more than 2000. The method was suitable for simultaneous analysis of OLME and CHLOR.

The concentration range of 10-200 µg/mL of OLME and 10-200 µg/mL of CHLOR were found to be linear with correlation coefficients 0.9996 and 0.9995 for OLME and CHLOR, respectively. The linearity curves of OLME and CHLOR are given in Fig. 3 and statistical parameters are shown in Table II. The LOD for OLME and CHLOR was found to be 0.566 µg/mL and 0.041 µg/mL, respectively and the LOQ was 1.71 µg/mL and 0.125 µg/mL for OLME and CHLOR, respectively. The results of recovery studies are shown in Table III. The average percent recovery for OLME and CHLOR was found to be 99.72% w/w and 100.56% w/w, respectively and the RSD% were less than 2.0%. The results show that the method is accurate

and suitable for assay of OLME and CHLOR in tablet dosage form. The results (Table IV) for repeatability and intermediate precision studies showed RSD values for peak response of both analytes to be less than 2.0. The results indicate that the given method is precise and repeatable within the acceptable limits and criteria. The results of robustness study showed that changes in the conditions like mobile phase pH (±2), flow rate (±0.1 mL) and mobile phase concentration (± 2 V/V) do not influence the results for OLME and CHLOR. The % RSD was less than 2.0 for all parameters (Table V), indicating that the method is robust and suitable for routine analysis assay of OLME and CHLOR in tablet dosage form.

There is no prominent peak of placebo (Fig. 2b) at the given retention time of OLME and CHLOR indicating no influence of tablet excipients in detection of OLME and CHLOR under the given chromatographic conditions. The results show that the proposed method is specific for quantification of OLME and CHLOR in dosage form. Stability of OLME and CHLOR in solution was investigated and the results are summarized in Table IV; the results show that the solutions are stable for 72 h at ambient temperature.

The method was successfully applied for assay of OLME and CHLOR from tablet dosage form. The percent content of OLME and CHLOR in tablet dosage form was found in the range of 99.12-100.48% for OLME and 98.62- 100.75% for CHLOR.

OLME and CHLOR were found to be relatively stable following photolysis and thermal degradation. Considerable degradation was observed for both on oxidation, acid and base hydrolysis. The validated method was applied to the determination of OLME and CHLOR in commercially available tablets. The results of the assay indicate that the method is selective for the analysis of both OLME and CHLOR without interference from the excipients used to formulate and produce these tablets. The method was applied for estimation of OLME and CHLOR in presence of degradation products of both drugs under variety of conditions. Hence, methods is stability indicating and can be used for quality control testing of marketed formulations. The chromatograms of OMLE, CHLOR and their degradation products for various stress conditions is enumerated in Fig. 4.

CONCLUSIONS

A simple stability-indicating RP-HPLC method has been developed and validated for the routine analysis of OLME and CHLOR in bulk and tablet dosage forms. The results of validation parameters are in complete agreement with the required limits and criteria. The proposed method has the ability to separate these drugs from their degradation products; excipients found in tablet dosage forms. The results of the forced degradation studies reveal that the method is stability indicating and can be applied to the analysis of samples. It can be concluded that the method is accurate, precise, linear, and specific for the simultaneous determination of OLME and CHLOR and can be applied to routine quality control analysis of OLME and CHLOR and establishment of stability data during formulation development.

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