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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND OLMESARTAN MEDOXOMIL IN TABLET DOSAGE FORM

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ABSTRACT

Keywords:

Metoprolol Succinate,
Olmesartan Medoxomil,
RP-HPLC, Validation

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A simple, selective and rapid reversed phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for the simultaneous analysis of metoprolol succinate and olmesartan medoxomil in tablet dosage form. The separation was carried out using a mobile phase consisting of 20mM phosphate buffer and Acetonitrile with pH 3.0 adjusted with ortho phosphoric acid in the ratio of 60: 40 % v/v. The column used was Thermo C18, (150 mm x 4.6 mm i.d, 5µm) with flow rate of 1 ml / min using PDA detection at 223 nm. The described method was linear over a concentration range of 0.5-25 µg/ml and 0.5-25 µg/ml for the assay of metoprolol succinate and olmesartan medoxomil respectively. The retention times of metoprolol succinate and olmesartan medoxomil were found to be 2.28 and 5.35 min respectively. Results of analysis were validated statistically and by recovery studies. The mean recovery was 99.60 ± 1.46 and 99.69 ± 1.31 for metoprolol succinate and olmesartan medoxomil, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) for Metoprolol Succinate and Olmesartan Medoxomil were found to be 0.137 and 0.416 µg/ml and 0.144 and 0.437 µg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of metoprolol succinate and olmesartan medoxomil in its pharmaceutical dosage form.

INTRODUCTION

Metoprolol succinate (METO) is chemically (*RS*)-1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol succinate^[1], is a cardio selective β -blocker, used in the treatment of hypertension, angina pectoris, arrhythmia, myocardial infraction and heart failure^[2]. It is official in IP^[3], BP^[4] and USP^[5]. IP^[3], BP^[4] and USP^[5] describes potentiometry method for its estimation. Literature survey reveals UV spectrophotometric method^[6], RP-HPLC method^[7], validated HPLC method for estimation of metoprolol in human plasma^[8], simultaneous spectrophotometric method with other drug^[9] and RP-HPLC method with other drug^[10] in pharmaceutical dosage forms as well as in biological fluids. Olmesartan medoxomil (OLME) is chemically (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1*H*-imidazole-5-carboxylate^[11], is a angiotensin II receptor antagonist for the treatment of hypertension^[12]. Olmesartan medoxomil is not official in any pharmacopoeia. Literature survey reveals stress induced method UV spectrometric method^[13], HPTLC^[14], simultaneous estimation with other drug^[15], HPTLC with other drug^[16], RP-HPLC with other drug^[17], stability-indicating LC method^[18] for the determination of OLME. The combined dosage forms of METO and OLME are available in the market for the treatment of hypertension. The present manuscript describes simple, sensitive, accurate, precise, rapid and economic chromatographic method (RP-HPLC) for simultaneous estimation of metoprolol succinate and olmesartan medoxomil in tablet dosage form.

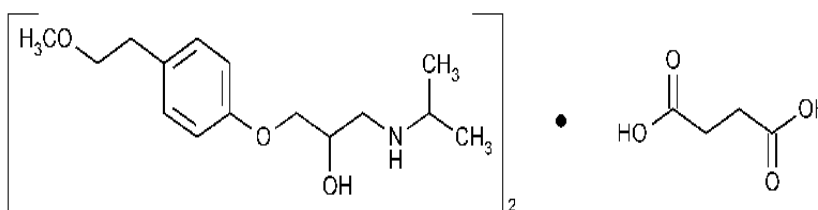


Fig. 1: Structural formula of metoprolol succinate

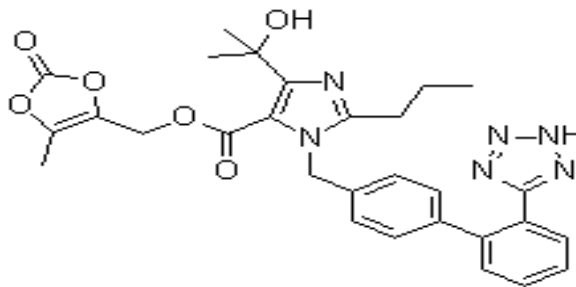


Fig. 2: Structural formula of olmesartan medoxomil

MATERIALS AND METHODS

Apparatus

RP-HPLC instrument (Shimadzu, LC-2010C_{HT}, Japan) equipped with a UV-Visible detector and a photodiode array detector, auto sampler, Thermo C₁₈ column (150 mm × 4.6 mm i.d., 5 µm particle size) was used. Chromatograms were automatically obtained by LC-solution system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

METO and OLME bulk powder was kindly gifted by Astron Research Centre, Ahmedabad, Gujarat, India. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from Finar Chemicals Ltd., Ahmedabad, India.

Preparation of standard stock solutions

An accurately weighed quantity of METO (10 mg) and OLME (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of METO (100 µg/ml) and OLME (100 µg/ml).

Preparation of Working Standard Solutions

An aliquot of stock solution 25 ml was transferred in 50 ml volumetric flask and adjusted up to mark with methanol having concentration (50 µg/ml)

Preparation of Sample Solution

Twenty tablets were weighed and powdered. The powder equivalent to 25 mg METO and 20 mg OLME was transferred to 100 ml volumetric flask. Methanol (50 ml) was added to it and sonicated for 20 min. The volume was adjusted up to the mark with methanol after filtration of sonicated solution.

Preparation of pH 3.0 Buffer Solution

Potassiumdihydrogen phosphate (20 mM, 2.72 gm) in 1000ml of millequivalent water was solubilised and adjusted the pH to 3.0 ±0.05 with ortho phosphoric acid solution.

Determination of Analytical Wavelength

The standard solution of METO and OLME were injected under the chromatographic condition described above. Detection was carried out at different wavelength best response was achieved at 223 nm with PDA detector. So both drugs were detected at this analytical wavelength (Fig. 3).

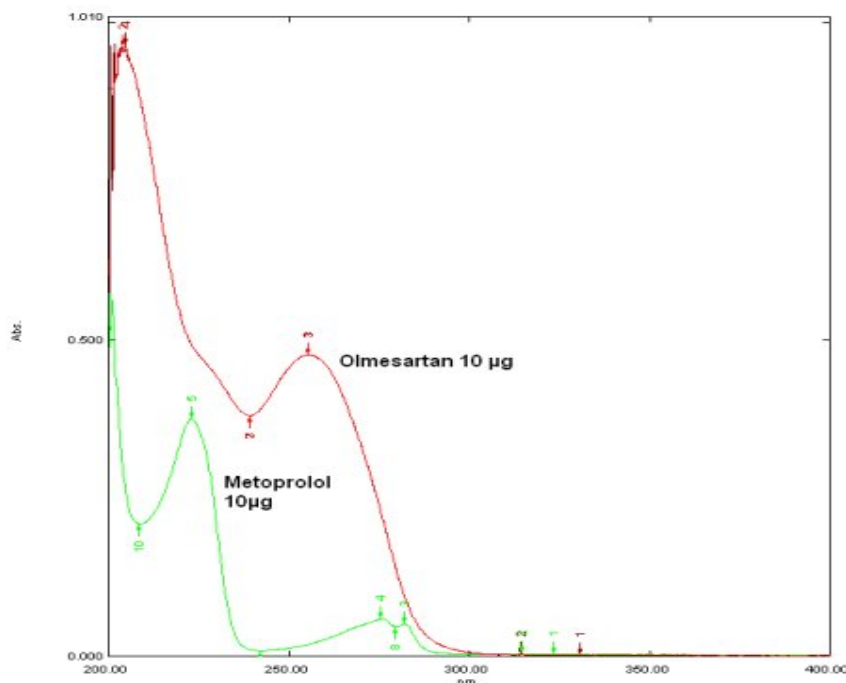


Fig. 3: Overlain zero-order absorption spectra of METO and OLME in methanol

Methodology

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for METO and OLME was obtained with a mobile phase Phosphate Buffer 20 mM (pH 3.0) : Acetonitrile [60:40 v/v] at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was carried out at 223 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Fig. 4). System suitability test parameters for METO and OLME for the proposed method are reported in Table 1.

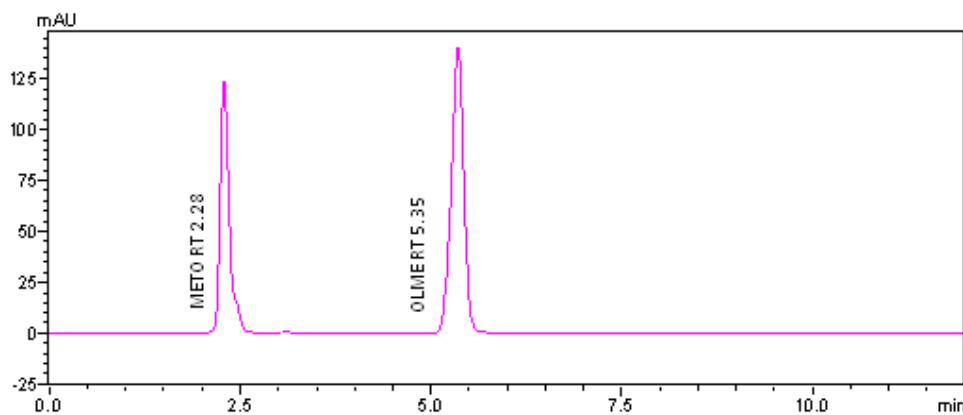


Fig.: 4 Chromatogram of Standard Solution of METO and OLME at 223 nm

TABLE 1: System Suitability Parameters of Chromatogram for METO and OLME

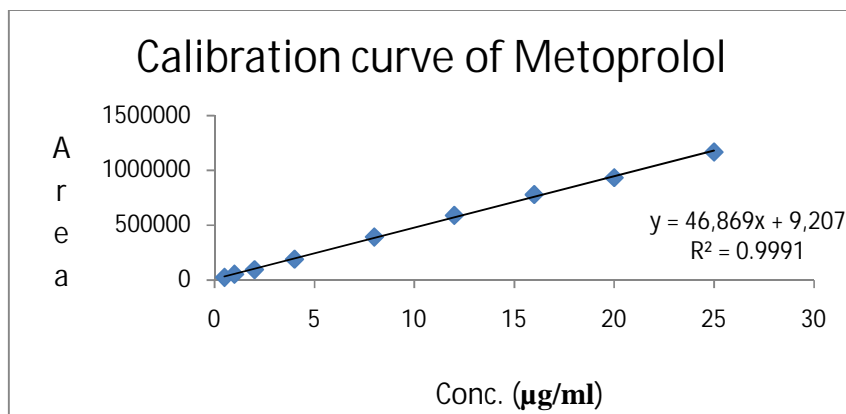
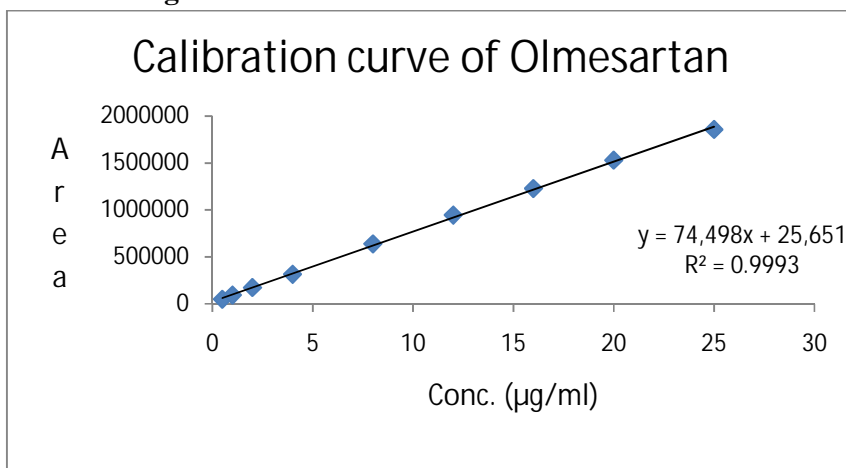
Parameters	METO \pm RSD (n = 6)	OLME \pm RSD (n = 6)
Retention time (min)	2.28 \pm 0.11	5.35 \pm 0.06
Tailing factor	1.23 \pm 1.44	0.84 \pm 0.99
Theoretical plates	2125 \pm 0.78	4948 \pm 0.51
Resolution	11.457 \pm 0.56	

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines^[19].

Linearity

Linear correlation was obtained between peak area Vs concentrations of METO and OLME in the concentration ranges of 0.5-25 $\mu\text{g/ml}$. Regression parameters are mentioned in table 4 and the calibration curves of these two drugs at 223 nm are shown in Fig. 5 & Fig.6.

**Fig: 5 Calibration Curve of METO at 223 nm****Fig: 6 Calibration Curve of OLME at 223 nm**

Method Precision (% Repeatability)

The RSD values for METO and OLME were found to be 0.64 and 0.63 %, respectively. The RSD values were found to be <2 %, which indicates that the proposed method is repeatable.

Intermediate Precision (Reproducibility)

The low RSD values of interday (0.36-1.08 % and 0.42-1.33 %) and intraday (0.56 – 1.57 % and 0.51 – 1.41 %) for METO and OLME, respectively, reveal that the proposed method is precise.

Limit of detection and Limit of quantification

Limit of detection (LOD) values for METO and OLME were found to be 0.137 µg/ml and 0.144 µg/ml, respectively and Limit of quantification (LOQ) values for METO and OLME were found to be 0.416 µg/ml and 0.437 µg/ml, respectively (Table 4). These data show that the proposed method is sensitive for the determination of METO and OLME.

Accuracy

The recovery experiment was performed by the standard addition method. The recoveries obtained were 100.1 ± 1.47 % and 99.79 ± 1.28 % for METO and OLME, respectively (Table 2). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2.

TABLE 2: Recovery Data for the proposed Method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery \pm SD*
METO	I	10	50 %	99.24 ± 1.41
	II	10	100 %	99.05 ± 1.92
	III	10	150 %	100.51 ± 1.05
OLME	I	8	50 %	99.25 ± 1.13
	II	8	100 %	100.82 ± 1.04
	III	8	150 %	98.99 ± 1.77

* Mean % Recovery \pm SD of six observations, SD = standard deviation

Assay of the Pharmaceutical Formulation

The proposed validated method was successfully applied to determine METO and OLME in their tablet dosage form. The result obtained for METO and OLME was comparable with the corresponding labeled amounts (Table 3). The RP-HPLC chromatogram for METO and OLME in sample was recorded and is shown in Fig. 7.

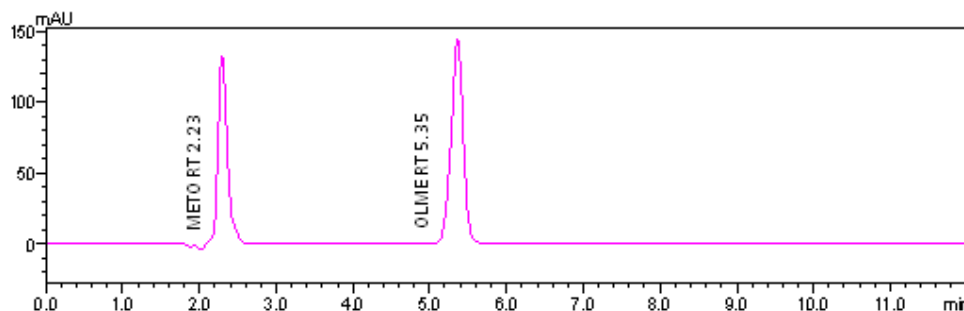


Fig: 7 Chromatogram of sample solution of METO and OLME at 223 nm

TABLE 3: Analysis of Formulation of Metoprolol and Olmesartan by Proposed Method (n = 6)

Tablet	Label claim (mg)		Amount found (mg)		% Label claim \pm SD (n=6)*	
	METO	OLME	METO	OLME	METO	OLME
I	25	20	24.87	19.87	99.48 \pm 0.777	99.35 \pm 1.488
II	25	20	24.95	20.02	99.83 \pm 0.687	100.10 \pm 0.140

*SD = standard deviation and n is number of determinations

RESULTS AND DISCUSSION

A RP-HPLC method was developed and validated for the determination of METO and OLME in tablet dosage forms on a column (C18, 150 X 4.6 i.d., 5 μ m) with variable wavelength detection at 223 nm. The retention times of METO and OLME was 2.28 min and 5.35 min, respectively.

Linear correlation was obtained between area and concentrations of METO and OLME in the concentration ranges of 0.5-25 μ g/ml and 0.5-25 μ g/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 4). The RSD values of were found to be 0.39 % and 0.42 of METO and OLME, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.36-1.08 % for METO and 0.42-1.33 % for OLME) and intraday (0.56 – 1.57 % for METO and 0.51 – 1.41% for OLME) at 223 nm, reveal that the proposed method is precise (Table 4). The limit of detection (LOD) and the limit of quantification (LOQ) for METO and OLME were found to be 0.137 and 0.416 μ g/ml and 0.144 and 0.437 μ g/ml, respectively. These data show that method is sensitive for the determination of METO and OLME.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.60 \pm 1.46 and 99.69 \pm 1.31 for METO and OLME, respectively (Table 4). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated

method was successfully applied to determine METO and OLME in their tablet dosage form. The results obtained for METO and OLME were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of METO and OLME in pharmaceutical dosage forms.

TABLE 4: Regression Analysis Data and Summary of Validation Parameter for the proposed Method

Parameters	RP-HPLC method	
	METO	OLME
Concentration range ($\mu\text{g/ml}$)	0.5-25	0.5-25
Slope	41687	67291
Intercept	17188	30308
Correlation coefficient	0.9991	0.9993
LOD ^a ($\mu\text{g/ml}$)	0.1376	0.1443
LOQ ^b ($\mu\text{g/ml}$)	0.4162	0.4373
% Recovery (Accuracy, $n = 6$)	99.60 \pm 1.46	99.69 \pm 1.31
Repeatability (% RSD, $n = 6$)	0.39	0.42
Precision (%RSD) ^c		
Interday ($n = 3$)	0.36-1.08	0.42-1.33
Intraday ($n = 3$)	0.56 – 1.57	0.51 – 1.41

^aLOD = Limit of detection. ^bLOQ = Limit of quantification. ^cRSD = Relative standard deviation.

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 1-10 $\mu\text{g/ml}$ with co-efficient of correlation, (r^2) = 0.9991 and (r^2) = 0.9993 for METO and OLME, respectively at 223 nm. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the METO and OLME in combined dosage form without any interference of excipients.

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