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Development and Validation of RP-HPLC Method for Simultaneous Determination of Metoprolol Succinate and Olmesartan Medoxomil in Bulk and Pharmaceutical Dosage Form

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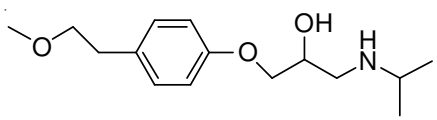
An accurate, precise and reproducible isocratic RP-HPLC method was developed and subsequently validated for the analysis of metoprolol succinate and olmesartan medoxomil in bulk and tablet dosage forms. Method development was carried out on Agilent Eclipse XBD-C18 (5 µm, 150 mm × 4.6 mm I.D.) column. The mobile phase was a mixture of acetonitrile and buffer (10 mM KH_2PO_4) in the ratio of 70:30 v/v. The pH of the buffer was adjusted to 2.75 with orthophosphoric acid. The flow rate was set at 0.6 mL/min and UV detection at 225 nm. The retention time of metoprolol succinate and olmesartan medoxomil were found to be 2.233 and 3.000 min, respectively. Validation parameters such as linearity, accuracy, precision and robustness, limit of detection (LOD) and limit of quantification (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. In the linearity study, the regression equation for metoprolol succinate and olmesartan medoxomil were found to be y = 68402x + 64710 and y = 110194x + 8855.3. Correlation coefficient was 0.9990 and 0.9996 for metoprolol and olmesartan, respectively. The proposed method is highly sensitive, precise, accurate, rapid and easy to perform and hence was successfully applied for the quantification of bulk and active pharmaceutical present in tablet dosage form.

Key Words: Metoprolol succinate, Olmesartan medoxomil, RP-HPLC, Validation, Simultaneous determination.

INTRODUCTION

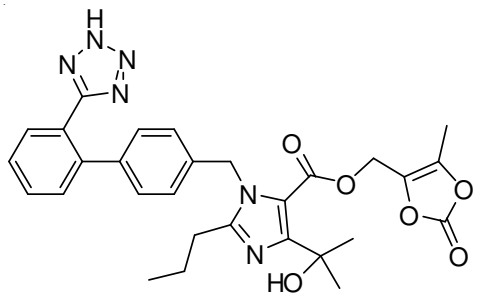
Metoprolol succinate (MET) is a selective β-adrenergic antagonist, which is used in the treatment of cardiovascular disorders such as hypertension, angina pectoris, cardiac arrhythmias, congestive heart failure and myocardial infarction[1-3]. It is used widely alone or in combination with other antihypertensive drugs for the treatment of cardiovascular disorders and cardiac failure[4-6].

International Union of Pure and Applied Chemist (IUPAC) name of metoprolol succinate (**I**) is 2-propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-butanedioate[7].



Metoprolol succinate (**I**)

Olmesartan medoxomil (**II**) is 5-methyl-2-oxo-1,3-dioxolen-4yl)methyl-4-(1-hydroxy-1-methylethyl)-2-propyl-1-[4-(2-(tetrazole-5-yl)phenyl]methylimidazole-5-carboxylate[8]. It used for the treatment of hypertension. It is a selective and competitive, non-peptide angiotensin II receptor antagonist; olmesartan medoxomil (OLM) blocks the vasoconstrictor and aldosterone secrecting effects of angiotensin II[9]. The drug contains a medoxomil ester moiety and is cleaved rapidly by an endogenous esterase to release the active metabolite olmesartan[10]. It is an approved drug for the treatment of hypertension in United States, Japan and European countries[11].



Olmesartan medoxmil (**II**)

Metoprolol succinate is official in the British Pharmacopoeia and United States Pharmacopoeia but olmesartan medoxomil is not. Their combination is also not official in Pharmacopoeia and no official method has been reported for their simultaneous assay[7,12].

A literature survey revealed spectrophotometric and HPLC methods for the separate determination of metoprolol succinate[13-17] and simultaneous determination of metoprolol succinate and some other drugs[18-22]. There are also many analytical methods for the determination of olmesartan medoxomil as a single drug[23-25] and methods for simultaneous determination of olmesartan medoxomil and some other drugs in pharmaceutical dosage forms as well as in biological fluids[26,27].

There is no literature information, to the best of our knowledge, on the simultaneous assay of metoprolol succinate and olmesartan medoxomil. This and the recent approval, by government of India for the use of metoprolol succinate and olmesartan medoxomil for combinational therapy in treatment of hypertension[28] prompted us to embark on this study. Thus, the goal of this study was to develop an HPLC method for simultaneous determination of metoprolol succinate and olmesartan medoxomil in pharmaceutical formulations with due consideration to rapidity, sensitivity, accuracy and economy.

EXPERIMENTAL

All solvents were of HPLC grade and reagents were of analytical grade. Acetonitrile was obtained from Sigma Aldrich, orthophosphoric acid and potassium dihydrogen orthophosphate (KH_2PO_4) were purchased from Rankem (Ranbaxy). Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a membrane filter (Millipore Millex®-FH, filter units, Durapore-PVDF, polyethylene, 0.22 µm pore size) and degassed before use. The active pharmaceutical ingredients, metoprolol succinate and olmesartan medoxmil were donated by Aurobindo Pharma Limited, Hyderabad, India and used as reference standards without further purification. Capsules of metolar containing 25 mg of metoprolol succinate manufactured by Cipla Pvt. Ltd. Tablets of olmesartan medoxomil containing 20 mg of olmesartan medoxmil (olmezst), manufactured by Sun Pharma Pvt. Ltd. and were purchased from market.

Chromatographic system: Chromatography was performed using a JASCO HPLC 2080 model chromatograph (Japan) equipped with a PU-2080 pump. The column used was an Agilent Eclipse XBD-reverse phase C_{18} column (150 mm × 4.6 mm I.D; particle size 5 µm) and detection was achieved using UV-2075 detector (JASCO). Data acquisition and processing was performed using JASCO BORWIN software (Japan). Sample injection was performed with a Rheodyne 7725 injection valve *via* a 20 µL loop. Dissolution of the compounds was enhanced by sonication on an ultrasonicator. UV spectra of metoprolol succinate and olmesartan medoxomil combination was taken using a JASCO V-550 UV-VIS spectrophotometer in order to select the working wavelength for detection of the drugs. All the weighing in the experiments was carried out on Digisum Electronic analytical balance (model DI 707).

All analyses were carried out at a column temperature of 22 ºC under isocratic conditions. The mobile phase was a mixture of acetonitrile and 10 mM potassium dihydrogen orthophosphate (pH adjusted to 2.75 with orthophosphoric acid) in the ratio of 70:30 v/v. The flow rate was 0.6 mL/min and volume of injection was 20 µL. UV detection was made at 225 nm.

Preparation of standard stock solution: The stock solutions were prepared by dissolving the suitable quantity of metoprolol succinate and olmesartan medoxomil to get the final concentration of 1.25 mg/mL and 1 mg/mL in standard volumetric flasks and made up the volume with acetonitrile. The working standard solution was made by diluting 10 mL of the respective standard stock solutions to 100 mL in volumetric flask to get 125 µg/mL of metoprolol succinate and 100 µg/mL of olmesartan medoxomil. Further dilutions were made from the working standard solution in the required concentration range in 10 mL volumetric flasks for the calibration curve.

Sample preparation: Synthetic mixture was prepared by taking individual dosage forms of 25 mg of metoprolol succinate and 20 mg of olmesartan medoxomil. Ten tablets were weighed and their average weight was calculated. The tablets were crushed to a blend homogeneous powder and a quantity equivalent to 100 mg was weighed into a 250 mL volumetric flask containing 150 mL of acetonitrile. This mixture was sonicated for 0.5 h and then filterd using Whatman filter paper No. 41. Finally, the solution was centrifuged and supernant was collected. Appropriate dilutions of the supernant were made with mobile phase to obtain different concentrations for analysis.

Optimization of mobile phase: A number of eluting systems were examined for optimization of the mobile phase for separation of the drugs, only mixture of acetonitrile and KH_2PO_4 buffer gave separation for the two drugs. Again, the mixture of acetonitrile and KH_2PO_4 buffer were screened as possible eluting systems in different proportions like 65:35, 60:40, 70:30 and 50:50 v/v. A mixture of acetonitrile and KH_2PO_4 buffer (pH adjusted to 2.75 with orthophosphoric acid), in the ratio of 70:30 v/v provided an efficient separation of the drugs with good peak shapes and retention times. A flow rate of 0.6 mL/min was found to be optimum and gave retention times 2.233 min for metoprolol succinate and 3.000 min for olmesartan medoxomil with baseline stability. A typical chromatogram is as shown on Fig. 1.

Method validation: Method validation was carried out according to the International Conference on Harmonization (ICH) guidelines[29-32]. Validation parameters examined were linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

Linearity: To establish linearity, stock solutions containing 125 µg/mL of metoprolol succinate and 100 µg/mL of olmesartan medoxomil were prepared by using acetonitrile. These were further diluted using mobile phase to get the solutions at the concentration range of 2.5-15 µg/mL and 2.0-12 µg/mL of metoprolol succinate and olmesartan medoxomil, respectively. Each of these drug solutions (20 µL) was injected into the chromatographic system. The peak area and retention

time were recorded and the mean values of peak areas were plotted against concentrations. The correlation coefficient, slope and Y-intercept values were calculated from the calibration plot obtained.

Limit of detection and limit of quantitation: Limit of detection is defined as the smallest level of analyte that gives a measurable response. Limit of detection is based on signal/noise (S/N) ratio typically for HPLC methods. Six replicates of the analyte were measured. The LOQ is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. The LOD and LOQ of metoprolol succinate and olmesartan medoxomil by the proposed methods were determined using calibration standards. Both LOD and LOQ values were calculated as 3.3 and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation.

System suitability: System suitability tests are used to verify that repeatability and resolution of critical parameter of system are adequate. The column efficiency, resolution and peak asymmetry were evaluated for the standard solutions. The values obtained demonstrated the system suitability for the analysis of two drugs and the system suitability parameters fell within ± 3 % standard deviation range during routine performance of the method.

Precision: The intra- and inter-day precision was determined by analyzing 10 µg/mL metoprolol succinate and 8 µg/mL olmesartan medoxomil, six times each on same day (intra-day study). This was repeated on the second day (inter-day study).

Accuracy: Recovery studies were performed by standard addition method. A known concentration of working standard was added to a fixed concentration of the pre-analyzed test solution. Three different levels corresponding to 8.0, 10.0 and 12.0 µg/mL for metoprolol succinate and 6.4, 8.0 and 9.6 µg/mL for olmesartan medoxomil were used in the studies. The analysis was conducted in triplicate. Percentage recovery was calculated by comparing the area before and after the addition of the working standard.

Robustness: The robustness of the developed method was determined according to ICH guidelines. Experimental conditions were deliberately altered one factor after the other. The effect of change in flow rate, organic phase composition, pH of buffer and column type on the retention time, peak asymmetry, theoretical plate number and resolution were studied.

RESULTS AND DISCUSSION

Typical chromatogram of metoprolol succinate and olmesartan medoxomil is shown in Fig. 1. By applying the proposed method, the retention times of metoprolol succinate and olmesartan medoxomil were found to be 2.233 and 3.000 min, respectively, showing that the proposed method is time saving one. The calibration curve showed linearity, over a concentration range of 2-15 µg/mL for metoprolol succinate and 2-12 µg/mL for olmesartan medoxomil (Figs. 2 and 3). Regression coefficient (R^2) was 0.9990 and 0.9996 metoprolol succinate and olmesartan medoxomil, respectively. Linear regression equations were y = 68402x + 64710 and y = 110194x + 8855.3 for metoprolol succinate and olmesartan medoxomil, respectively, where y is the peak area and x is concentration of metoprolol succinate and olmesartan medoxomil, (Table-1). The number of theoretical plates obtained was 2970 and 3469 for metoprolol succinate and olmesartan medoxomil, respectively which indicates the efficient performance of the column. The LOD and LOQ were found to be 0.052, 0.158, 0.034 and 0.102 ppm for metoprolol succinate and olmesartan medoxomil, respectively, which indicates the high sensitivity of the method (Table-2).

The results of intra-day and inter-day precision are as presented in Table-3. Assay of the two drugs using the developed method showed acceptable relative error values. The RSD % for assay of drugs during intra-day and inter-day were 0.756 and 1.024 for metoprolol succinate and 0.996 and 1.077 for olmesartan medoxomil.

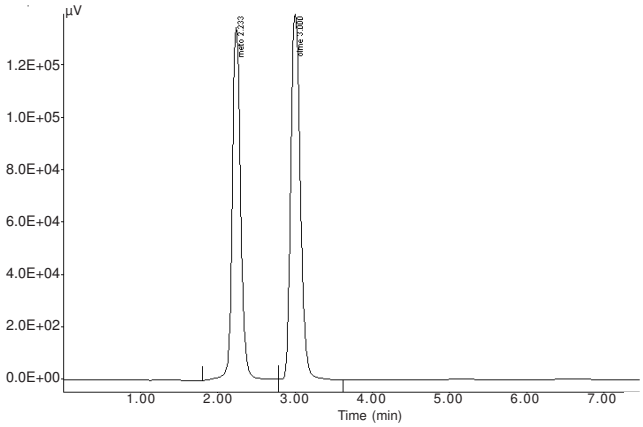


Fig. 1. Typical chromatogram of metoprolol succinate and olmesartan medoxmil

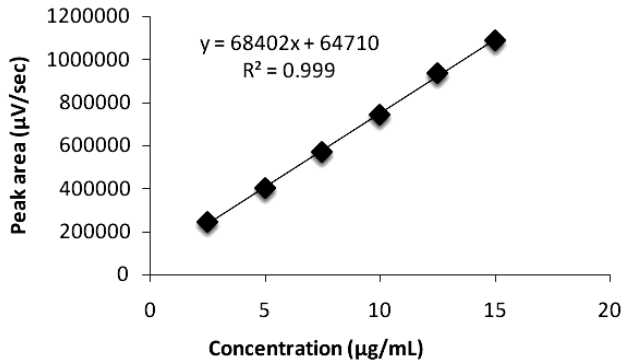


Fig. 2. Linearity curve of metoprolol succinate

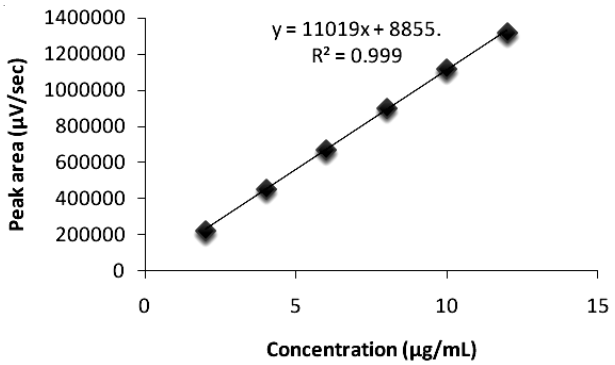


Fig. 3. Linearity curve of olmesartan medoxmil

TABLE-1
ANALYTICAL CHARACTERISTICS OF THE PROPOSED METHOD DERIVED FROM THE STANDARD CALIBRATION CURVE

Parameters	Metoprolol succinate	Olmesartan medoxmil
Slope	68402	110194
Intercept	64710	8855.3
Equation of regression	y = 68402x + 64710	y =110194x+8855.3
Correlation coeffficient	0.999	0.9998
LOD (µg/mL)	0.052	0.034
LOQ (µg/mL)	0.158	0.102

TABLE-2
SYSTEM SUITABILITY PARAMETERS

Parameters	Metoprolol succinate	Olmesartan medoxomil
Retention time (min)	2.233	3.000
Resolution	–	4.06
Theoretical plate No.	2970	3469
Peak asymmetry	1.21	1.18
LOD (µg/mL)	0.052	0.034
LOQ (µg/mL)	0.158	0.102

The percentage mean recovery at three different levels of study were 99.5 ± 0.23, 98.5 ± 0.08 and 100.3 ± 0.18 for metoprolol succinate and 99.1 ± 0.03, 100.4 ± 0.02 and 99.80 ± 0.05 for olmesartan medoxomil (Table-4). The percentage mean recovery of individual analyte was high, satisfactory and indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations didn't interfere with the estimation of the drug by the proposed HPLC method.

In robustness study, four factors (retention time, pH of buffer, composition of buffer and effect of column) were deliberately altered. In all these, the resolution between metoprolol succinate and olmesartan medoxomil, peaks was greater than 4.0, asymmetric factor was less than 2.0 and theoretical plates were more than 2800 for metoprolol succinate and olmesartan medoxomil peaks, which illustrates the good robustness of the developed method (Table 5a-b).

Conclusion

The proposed isocratic RP-HPLC method was found to be simple, fast, precise and accurate. The ease of preparation of mobile phase, well separated peaks, good resolution, run time of 3 min and its cost effectiveness make the proposed method adequate enough for routine simultaneous analysis of metoprolol succinate and olmesartan medoxomil in laboratories and pharmaceutical industries.

TABLE-3
RESULTS OF PRECISION STUDY (n = 6)

Drug	Concentration (µg/mL)	Intra-day precision		Inter-day precision	
		Found concentration ± SD; RSD (%)	Found (%)	Found concentration ± SD; RSD %	Found (%)
Metoprolol succinate	10	9.929 ± 0.075; 0.756	99.3	9.928 ± 0.102; 1.024	99.28
Olmesartan medoxomil	8	8.017 ± 0.077; 0.966	100.2	8.086 ± 0.087; 1.077	101.08

TABLE-4
RESULTS OF ACCURACY STUDIES (n = 3)

Analyte	Amount (%) of drug added to the analyte	Theoretical conc. (µg/mL)	Measured conc. (µg/mL) ± SD	Recovery (%)	RSD (%)
Metoprolol succinate	80	8	7.96 ± 0.23	99.50	0.28
	100	10	9.85 ± 0.08	98.45	0.77
	120	12	12.03 ± 0.18	100.26	1.48
Olmesartan medoxmil	80	6.4	6.34 ± 0.03	99.13	0.45
	100	8	8.03 ± 0.07	100.36	0.87
	120	9.6	9.58 ± 0.05	99.80	0.52

TABLE-5
(a) Evaluation data of robustness study for metoprolol succinate

Robustness conditions	System suitability parameters			
	Retention time (min)	Peak asymmetry	Theoretical plate No.	Resolution
Flow-rate (0.5 mL/min	2.381	1.22	3018	–
Flow-rate (0.7 mL/min	2.102	1.18	2892	–
pH 2.7	2.295	1.20	3112	–
pH 2.9	2.117	1.22	2824	–
Acetonitrile-KH_2PO_4 (69:31, v/v)	2.243	1.20	2865	–
Acetonitrile-KH_2PO_4 (71:29, v/v)	2.145	1.22	2984	–
Column change	2.345	1.18	3124	–
(b) Evaluation data of robustness study for Olmesartan medoxmil				
Flow-rate (0.5 mL/min	3.201	1.18	3612	4.04
Flow-rate (0.7 mL/min	2.972	1.16	3345	4.07
pH 2.7	3.334	1.16	3586	4.09
pH 2.9	2.916	1.19	3320	4.06
Acetonitrile-KH_2PO_4 (69:31, v/v)	3.176	1.16	3352	4.10
Acetonitrile-KH_2PO_4 (71:29, v/v)	2.940	1.19	3587	4.06
Column change	3.120	1.18	3692	4.11

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