RAPID RP-HPLC METHOD FOR SIMULTANEOUS SEPARATION AND ESTIMATION OF METOPROLOL AND AMLODIPINE IN COMBINATION TABLET FORMULATION

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ABSTRACT

The aim of the present work was to develop a RP-HPLC method for simultaneous separation of Metoprolol and Amlodipine and validate the developed method for quantitative determination of the above said drugs in combination tablet dosage form. A simple, rapid, validated chromatographic method was developed for separation and determination of metoprolol and amlodipine in tablet preparations. The anti-hypertensive agents were separated using Welchrom RP-C₁₈ Column (4.6 mm X 250 mm, 5 μ), Shimadzu LC-20 AT Prominence Liquid Chromatograph and the mobile phase constituted of 10 mM Phosphate buffer (pH 3.0): acetonitrile (50:50, v/v). The flow rate was 1.0 mL/min and the analyses were performed using Shimadzu SPD-20A Prominence UV-Visible detector at 235 nm. The separation of the anti-hypertensive agents, Metoprolol and Amlodipine was achieved within 5 minutes. Metoprolol and Amlodipine eluted at retention times of 2.690 minutes and 3.797 minutes respectively. The drugs obeyed Beer's law in the concentration range of 5 - 25 μ g/mL for metoprolol and 1 - 5 μ g/mL for amlodipine. The developed assay method was found to be selective, precise and accurate. The method has been successfully applied for determination of Metoprolol and Amlodipine in pharmaceutical combination tablet dosage form. This developed method is sensitive, fast, and simple with excellent peak symmetry and high resolution.

Keywords: Metoprolol, Amlodipine, Isocratic RP-HPLC-UV method, Validation.

INTRODUCTION

Metoprolol succinate (MET) is used alone or in combination with other medicines to treat high blood pressure. It also used to prevent angina and to improve survival after a heart attack. Extended-release (long-acting) MET is also used in combination with other medications to treat heart failure. It works by relaxing the blood vessels and slowing heart rate to improve blood flow and decrease blood pressure. MET belongs to class of medications called beta blockers. It is chemically 1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]-2-propanol succinate (Figure 1). Amlodipine besylate (AML) is a calcium channel blocker belonging to the class of 1, 4-dihydropyridines. They are potent dilators of peripheral and coronary arteries and extensively and primarily used in the treatment of hypertension and is also used in the prophylaxis of angina. It can be used alone or in combination with other drugs like adrenergic blocking agents, ACE inhibitors or diuretics to treat hypertension. The usual dose of AML is 5 mg/day. It can be increased upto 10 mg/day depending on individual's response. Major side effects of AML are slow heartbeat, shortness of breath, fainting, dizziness etc., AML is chemically 3-ethyl-5-methyl-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate (Figure 1). Literature survey reveals that for the determination of above said anti-hypertensive agents in biological fluids like plasma, blood, urine and pharmaceutical dosage forms by HPTLC¹, High Performance Liquid Chromatography (RP-HPLC)²⁻⁵ and Liquid Chromatography-Mass Spectrometry⁷⁻⁹, spectrofluorimetry¹⁰. The available methods are either poorly validated or uneconomical. In fact there is a need for the development of a novel, simple, rapid, efficient RP-HPLC analytical method with reproducibility for determination of MET and AML in pharmaceutical dosage forms. Thus, the proposed method provides rapid separation with effective resolution, excellent peak shape, use of smaller sample volumes and buffer volumes, providing cost savings. The

established method was validated with respect to specificity, linearity, precision, accuracy, robustness, LOD and LOQ according to ICH guidelines¹¹.

MATERIALS AND METHODS

Chemicals and Reagents

The reference standards of MET and AML were gifted by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate was purchased from Rankem Ltd., Mumbai, India, while HPLC grade Acetonitrile and triethylamine from Merck Pharmaceuticals Private Ltd., Mumbai, India. O-Phosphoric acid (HPLC grade) was purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets containing MET (25 mg) and AML (2.5 mg) was procured from local market. MOTOBLOCK-AM tablets are manufactured by Talent Healthcare Ltd., Sidcul-Haridwar, India.

Instruments and Chromatographic conditions

Chromatographic separations were achieved by using Shimadzu LC-20AT Prominence Liquid Chromatograph comprising a LC-20AT VP pump, Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C₁₈column (4.6 mm i.d. X 250 mm, 5 μ particle size). The volume of sample injected was 20 μ L using manual syringe injector. The HPLC data acquisition was achieved with "Spinchrom" software. Separations were accomplished on the reversed phase column using a mixture of 10 mM Phosphate buffer (pH adjusted to 3.0 using triethylamine) and Acetonitrile in ratio of 50:50, % v/v as mobile phase. The mobile phase was delivered at a flow rate of 1 mL/min. Eluate was detected at 235 nm. In addition, an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), an Ultrasonicator, UV-Visible Spectrophotometer (Systronics model 2203) were used in this study.

Preparation of Reagents and Standards

Mobile phase

Accurately weigh and dissolve 6.056 g of potassium dihydrogen orthophosphate in 445 mL of HPLC grade water. To this, 55 mL of 0.1M phosphoric acid is added and mixed well to get 10 mM Phosphate buffer. The pH of the solution is then adjusted to 3.0 with triethylamine. The above prepared buffer and acetonitrile were mixed in the ratio of 50:50, % v/v. The mobile phase was then filtered through 0.45 µm nylon membrane vacuum filtration and degassed by sonication.

Preparation of Standard Stock Solutions

A standard stock solution of the drug was prepared by dissolving 50 mg of MET and 5 mg of AML in 100 mL volumetric flask containing 70 mL mobile phase, then sonicated for about 10 minutes and made upto 100 mL with mobile phase to get the primary standard stock solution containing 500 µg/mL of MET and 50 µg/mL of AML.

Working standard solution

1 mL of the above stock solution was taken in 10 mL volumetric flask and made upto 10 mL with mobile phase to get the working standard solution containing 50 µg/mL of MET and 5 µg/mL of AML. Then, aliquots of working standard solution are then suitably diluted to give the calibration solutions containing metoprolol and amlodipine in the concentration ranges of 5, 10, 15, 20, 25 µg/mL and 1, 2, 3, 4, 5 µg/mL respectively.

Preparation of Sample solution

20 tablets of MOTOBLOCK-AM were weighed and powdered. A sample of powdered tablets equivalent to 50 mg of MET and 5 mg of AML was mixed with 100 mL mobile phase. The mixture was allowed to stand for 30 minutes with intermittent sonication to ensure complete dissolution. Filter through a 0.45 µm membrane filter. Pipette out and dilute the solution with mobile phase to get a final concentration of 12.5 µg/mL of MET and 1.25 µg/mL of AML.

Selection of detection wavelength

The superimposed UV spectra of various diluted solutions of MET and AML in mobile phase were noted using UV spectrophotometer. The isobestic point of highest absorbance was noticed at 235 nm. This wavelength was used for detection of MET and AML.

Validation of the Developed Method

The developed method of analysis was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, precision, accuracy and system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability

System suitability was ascertained by six replicate analyses of the drugs at concentrations of 25 µg/mL of MET and 5 µg/mL of AML respectively. The % RSD of the peak areas and retention time for the MET and AML are within the limits of less than 2 % which indicates the suitability of the system. The system suitability parameters like resolution (NLT 2.0), tailing factor (NMT 1.5), theoretical plate count

(NLT 3000) and % RSD for peak area of six replicate injections of standard (% RSD NMT 2.0) are within the limits. The results for system suitability are presented in Table 1.

Specificity

The specificity of the method is performed by separate injections of MET and AML standard and sample. The retention time of the MET does not interfere with retention time of AML. The effect of wide range of excipients and other additives usually present in the formulations in the determinations under optimum conditions was investigated. The common excipients such as lactose anhydrous, microcrystalline cellulose, purified talc, hydroxyl propyl methyl cellulose (HPMC) and magnesium stearate have been added to the placebo solution and injected and tested. The chromatogram for placebo indicating the specificity of developed method is presented in Figure 2. The specificity results are tabulated in Table 2.

Linearity

Aliquots of primary working standard solution containing MET and AML were diluted such a way that the final concentrations of MET and AML are in the range of 5 - 25 µg/mL and 1 - 5 µg/mL respectively. The linearity graphs for the proposed assay methods were plotted over the concentration range. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data values. A calibration curve was plotted between concentration and peak area response and statistical analysis of the calibration curve was performed.

Precision

Precision was determined by intra-day and inter-day study. Precision of the method was evaluated by carrying out the assay and analyzing corresponding responses 6 times on the same day and on different days for the sample solution. The percent relative standard deviation (% RSD) was calculated.

Accuracy (Recovery studies)

Accuracy studies were performed for both MET and AML at three different levels (80 %, 100 % and 120 %) and the mixtures were analyzed in triplicate by the proposed method. Known amount of standard MET and AML at 80 %, 100 % and 120 % of predetermined sample was added to a pre quantified tablet sample and analysed in triplicate.

LOD and LOQ

Limit of Detection and Limit of Quantitation were calculated using following formula $LOD = 3.3(SD)/S$ and $LOQ = 10 (SD)/S$, where SD = standard deviation of response (peak area) and S = slope of the calibration curve. The LOD and LOQ for the estimation of MET was found to be 0.9778 µg/mL and 2.9632 µg/mL and for the estimation of AML were 0.1859 µg/mL and 0.5635 µg/mL respectively.

ASSAY

20 µL of sample solution was injected into Liquid Chromatograph. The assay was repeated for six times and the amount of the drug present per tablet was determined from calibration equation. The mean % recovery was determined.

Table 1: Optimized Chromatographic Conditions and System Suitability Parameters for the Proposed Method

Parameter	Chromatographic conditions	
Instrument	SHIMADZU LC-20AT Prominence liquid chromatograph	
Column	WELCHROM C ₁₈ Column (4.6 X 250 mm, 5 µm)	
Detector	SHIMADZU SPD-20A Prominence UV-Vis detector	
Diluents	10 mM Phosphate Buffer (pH 3.0) : Acetonitrile (50:50, v/v)	
Mobile phase	10 mM Phosphate Buffer (pH 3.0) : Acetonitrile (50:50, v/v)	
Flow rate	1 mL/min.	
Detection wave length	UV at 235 nm.	
Run time	5 minutes	
Column back pressure	155-158 kgf	
Temperature	Ambient temperature (25°C)	
Injection Volume	20 µL	
	Metoprolol succinate	Amlodipine besylate
Retention time (t _R)	2.690 minutes	3.797 minutes
Theoretical plates [th.pl] (Efficiency)	8672	10160
Resolution	-	8.331
Tailing factor (asymmetry)	1.4628	1.2236

Table 2: Specificity Study

Name of the solution	Retention time, (t _R) minute
Mobile phase	No peaks
Placebo	No peaks
Solution containing a concentration of MET, 25 µg/mL and AML, 5 µg/mL.	Peaks at 2.690 minutes and 3.797 minutes for MET and AML respectively.

Table 3: Linear Regression Data

Parameter	Metoprolol	Amlodipine
Detection wavelength (λ _{max})	UV at 235 nm	UV at 235 nm
Linearity range (µg/mL)	5 - 25 µg/mL	1 - 5 µg/mL
Regression Equation (Y = aX + b)	Y = 48.639X + 15.360	Y = 83.640X + 3.421
Slope (a)	48.639	83.640
Intercept (b)	15.360	3.421
Standard error of slope (S _a)	0.689065	1.126637
Standard error of intercept (S _b)	10.431246	3.411062
Standard error of estimation (S _e)	14.412838	4.713060
Regression coefficient (R ²)	0.9992	0.9993
% Relative standard Déviation* i.e., Coefficient of variation (CV)	1.8924	1.6971
Percentage range of errors (Confidence limits)		
0.005 significance level	1.020866	1.937574
0.001 significance level	1.601302	3.039224

*Average of 6 determinations; acceptance criteria < 2.0

Table 4: Calibration Data

Metoprolol		Amlodipine	
Concentration, µg/mL	Peak area, mV.s.	Concentration, µg/mL	Peak area, mV.s.
0	0	0	0
5	264.374	1	87.126
10	518.716	2	175.76
15	741.639	3	252.725
20	997.324	4	342.885
25	1217.993	5	416.632

Table 5: Results of Precision Study

Precision Study	Metoprolol	Amlodipine
	% RSD [#]	% RSD [#]
Intra-day	0.4876	0.9058
Inter-day	0.5331	1.1176

*Acceptance criteria < 2.0

Table 6: Recovery Data

Recovery level [#]	Metoprolol	Amlodipine
	Mean % Recovery $\pm$ SD	Mean % Recovery $\pm$ SD
80 %	100.353 $\pm$ 0.749	100.365 $\pm$ 1.008
100 %	99.654 $\pm$ 0.471	100.588 $\pm$ 0.530
120 %	100.811 $\pm$ 0.845	99.984 $\pm$ 1.107

[#] average of triplicate injections

Table 7: Assay Results

Formulation	Labeled amount		Amount found		% Assay $\pm$ SD*	
	MET	AML	MET	AML	MET	AML
Motoblock-AM tablets (Talent Healthcare Ltd., Sidcul-Haridwar, India)	25 mg	2.5 mg	24.843 mg	2.534 mg	99.375 $\pm$ 1.912 %	101.398 $\pm$ 1.706 %

*Average of 6 determinations; SD is standard deviation

Figure 1: Structures of Anti-Hypertensive drugs investigated in the present study

Figure 2: Chromatogram of placebo

Figure 3: Standard chromatogram of Metoprolol succinate (25 µg/mL) and Amlodipine besilate (5 µg/mL)

Figure 4: Calibration plot of Metoprolol succinate

Figure 5: Calibration plot of Amlodipine besilate

Figure 6: Chromatogram of the sample of market formulation (Motoblock-AM tablets)

RESULTS AND DISCUSSION

For obtaining suitable mobile phase for the analysis of the selected drug combination, various mixtures of acetonitrile and phosphate buffer were tested. After some trials, it was found that the mixture of phosphate buffer (pH-3.0) and acetonitrile in a composition of 50:50, % v/v as mobile phase resulted in symmetric peak at 235 nm in short runtime (5 minutes). The pH of buffer was corrected to 3.0 using triethylamine. Various column types and lengths were tried regarding other chromatographic parameters. C_{18} column with a 4.6 mm inner diameter, 250 mm length and 5 micron particle size was preferred. UV overlain spectra of these drugs showed that these drugs absorbed appreciably at 235 nm, so that this wavelength was chosen as the detection wave length. Flow rate used was set to 1 mL/min. chromatograms showed a peak of MET at retention time of 2.690 minutes and peak of AML at retention time of 3.797 minutes respectively. The calibration curve was obtained for a series of concentration in the range of 5 - 25 $\mu\text{g/mL}$ for MET and 1 - 5 $\mu\text{g/mL}$ for AML respectively and it was found to be linear. The data of regression analysis of the calibration curve is depicted in Table 3. The calibration data is presented in Table 4. The regression equation obtained from linearity plot for MET was $Y = 48.639X + 15.360$ with $R^2 = 0.9992$ and for AML was $Y = 83.640X + 3.421$ with $R^2 = 0.9993$ which indicates this method had good linearity. The representative standard chromatogram is shown in Figure 3. The calibration plot for MET and AML were shown in Figure 4 and Figure 5 respectively. The proposed method was found to be precise for the determination MET and AML. The % RSD for the proposed method was found to be less than 2.0 which indicate the method's precision. Results of the precision study are shown in the Table 5. Recovery studies (Table 6) of the method was found to be good within the overall mean % recovery of the tablet dosage form. The developed method was applied to the assay of Motoblock-AM tablets and results are shown in Table 7. The representative sample chromatogram is shown in Figure 6. The assay results of different injections of the sample were found to be within the proposed limits. The mean assay value was found to be $99.375 \pm 1.912\%$ for MET and $101.398 \pm 1.706\%$ for AML.

CONCLUSION

The present method demonstrated the estimation of MET and AML available as combination tablet dosage forms using RP-HPLC. The linearity of the proposed method was in the range

of 5 - 25 $\mu\text{g/mL}$ for MET and 1 - 5 $\mu\text{g/mL}$ for AML respectively. The developed RP-HPLC method for the quantification of MET and AML was found to be simple, specific, highly sensitive, rapid, economical, precise and very accurate. The developed method has various advantages like good linearity, smaller retention times and low solvent consumption which make the method economical. So, the developed method may be successfully applied for the simultaneous determination of MET and AML in pharmaceutical quality control laboratories for routine analysis.

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