Research Article

Simultaneous Estimation of Motoprolol and Amlodipine Besylate

Satyajeet Singh, Umesh Galotra, Jai Prakash Kadian and Rajeev Tomer

Roorkee College of Pharmacy Kishanpur Roorkee, India.

ABSTRACT

A new UV- Spectrophotometric method has been developed for the simultaneous estimation of metoprolol and amlodipine besylate in tablet dosage forms using 0.1N hydrochloric acid buffer (pH 1.2). The method is based on simultaneous equation or Vieordt's method. The λ_{max} values for atenolol and amlodipine besylate were found to be 224.6 nm and 239.6 nm respectively. The system obey Beer's law in the range of 4-28 µg/ml and 4-32 µg/ml with correlation coefficient of 0.9991 and 0.993 for metoprolol and amlodipine besylate respectively. Intraday and Interday precision were found to be 0.08577-1.4682, 0.1080-1.71138, 0.2525-1.6080 and 0.2599-1.3906 respectively. The developed method can be successfully employed for the assay of metoprolol and amlodipine besylate in different formulations.

Keywords: Metoprolol, Amlodipine besylate, UV-Spectrophotometry, Vierodt's method.

INTRODUCTION

Metoprolol (MOTO) chemically 1-(isopropyalamino)3-(4-(2-methoxyethyl) phenoxy)propan-2-ol is a β-adrenoreceptor blocking agent primarily used for hypertension, angina pectoris & myocardial infarction. It mainly acts by inhibition of rennin release and angiotension – II (AT-II) and aldosterone production[1,4].

Amlodipine besylate (AML) chemically 3-Ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate is a long acting calcium channel blocker used for hypertension and angina pectoris. Amlodipine besylate block the inward movement of calcium by binding to L-Type calcium channels in the heart and in smooth muscle and dilating arterioles thereby decreasing peripheral resistance. Hence improving blood pressure; in angina it improves blood flow to the myocardium[1,4].

In, the present study 0.1N hydrochloric acid buffer (pH 1.2) is used as solvent for simultaneous estimation of both the drugs by simultaneous equation or Vierodt's method using UV-Spectrophotometry. The present method is relying on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC & HPTLC.

METHOD AND MATERIAL

Instrument

Shimazdu-1700 Double beam UV-VIS Spectrophotometer with spectral band width of 1.8nm, wavelength accuracy of ±2nm and matched quartz cells of 10mm optical path length was used for all spectral and absorbance measurements.

MATERIALS

Metoprolol and Amlodipine besylate was obtained as a gift sample from Alembic Pharma, Mumbai, India. All chemicals and buffers were of analytical grade.

Preparation of stock solution of Metoprolol and Amlodipine besylate

A standard stock solution of MOTO and AM were prepared by dissolving 100mg in 100ml 0.1N hydrochloric acid buffer(pH 1.2) and further 1ml is pipette out and diluted to 100ml with 0.1N hydrochloric acid buffer (pH 1.2). Different dilutions were prepared to get working concentrations of 4-28 and 4-32 For MOTO and AM respectively. The aliquots of pure MOTO and AM were transferred

into a calibrated flasks and total volume was adjusted upto mark with 0.1N hydrochloric acid buffer (pH 1.2). The absorbance's of the resulting solution were then measured at 224.6 nm and 239.6 nm respectively and calibration curves were plotted between absorbance v/s concentrations.

Accuracy

As a part of determining accuracy of the proposed method, different levels of drug concentrations (LQC, MQC and HQC) were prepared from the independent stock solution[7].

Precision

Intraday and interday variations were taken to determine intermediate precisions of the proposed method. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intraday variations. The same drug concentrations were prepared on three different days to study interday variations. The coefficient of variations (%) of the predicted concentrations from the regression equations was taken as precision[7].

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD was determined using the relation 3.3 σ/s where 'σ' is the standard deviation of the response and s is the slope of the calibration curve[9,6].

Similarly, LOQ was determined using the relation 10 σ/s.

Bench top stability study

In this method stock solution stability was determined by preparing LQC, MQC and HQC at different intervals of time[9,6].

Assay of Formulations

Twenty tablets each of two brands were weighed and ground in to a fine powder. Powder equivalent to 25mg and 5mg of MOTO and AM was transferred into 100 ml volumetric flaks and dissolved in 25 ml of 0.1N hydrochloric acid buffer (pH1.2). The solution was sonicated for 20minuted and was filtered through whatmann No.40 filter paper. The residue was washed with hydrochloric acid buffer and washings were added to filterate. The volume was made upto the mark with 0.1N hydrochloric acid buffer so as to get a concentration of 250.0 µg/ml of metoprolol and 500 µg/ml. From this solution, (1.5ml) was pipette out into 10 ml volumetric flask and diluted upto the mark with 0.1N hydrochloric acid buffer (pH 1.2) so as to get a concentration of 80.0 µg/ml and 8.0 µg/ml for amlodipine besylate. The absorbances of these solutions were measured at 224.6 nm and 239.6 nm using 0.1 N hydrochloric acid buffer (pH 1.2).

RESULT AND DISCUSSION

Analytical data

A linear correlation was found between absorbances at λ_{max} and concentrations of MOTO and AM. The optical characterstics such as Beer's law limits, molar absorptivity values were given in Table1. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values are reported in Table 1. The graph shows negligible intercept as described by the regression equation Y = a + bX where Y is the absorbance and X concentration in µg/ml. the limit of detection and quantification calculated according to ICH guidelines and reveals a very high sensitivity of the methods[8].

METHOD VALIDATION

Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solutions at three different levels (within working limits) were analyzed, each determination being repeated three times. The relative standard deviation (%) were in range of 0.08527-1.4682 for metoprolol (intraday), 0.1080-1.7118 for metoprolol (intraday), 0.2525-1.6080 for amlodipine besylate (intraday) and 0.2599-1.3906 for amlodipine besylate (interday) respectively. These values were significant and can be used for routine analysis.

Application to analysis of commercial samples

In order to check the validity of the proposed method, MOTO and AM were determined in some commercial formulations. Table 2 presents the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the labeled claim.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powders were spiked with pure MOTO and AM standard solutions at three different levels and the concentrations of the sum total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug solution was quantitative (98.14-99.08% and 99.06-99.52% respectively) and reveals that co-formulated substances did not interfere in the determination.

CONCLUSION

The proposed methods for determination of metoprolol and amlodipine besylate have been developed and validated. These are applicable over a range of 4-28 µg/ml for ATE and 4-32 µg/ml for AM and molar absorptivity of 402.50 L mol^{-1} cm^{-1} for MOTO and 297.00 L mol^{-1} cm^{-1} for AM. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC, HPTLC. Thus these can be used as alternatives for rapid and routine determination of bulk samples and tablets.

MATHEMATICAL CALCULATIONS

Amounts of metoprolol and Amlodipine besylate were determined by solving the simultaneous equations. Two simultaneous equations were formed using absorptivity coefficient values.

$$A_1 = 402.50 \times C_1 + 38.84\ C_2$$
$$A_2 = 297.00 \times C_1 + 32.22\ C_2$$

Where C_1 and C_2 are the concentrations of Metoprolol and Amlodipine besylate respectively in gm/liter in the sample solution, A_1 and A_2 are the absorbances of the mixture at the 224.6 nm and 239.6 nm respectively.

ACKNOWLEDGEMENTS

The authors are thankful to Alembic Pharma, Mumbai, India for the gift sample (Metoprolol and Amlodipine besylate) and Director, Roorkee College of Pharmacy Roorkee for providing facilities to carry out this work.

Table 1: Result of Analytical method Development

Parameter	Metoprolol at 224.6nm	Amlodipine Besylate at 239.6 nm	Metoprolol at 239.6nm	Amlodipine Besylate at 224.6nm
Beer's law Limit (µg/ml)	4-28	4-32	50-350	50-350
Absorptivity (L mol^{-1} cm^{-1})	402.50	297.00	38.84	32.22
Regression equation				
Slope	0.040	0.041	0.002	0.003
Intercept	0.003	0.015	0.058	0.017
Correlation coefficient(r^2)	0.999	0.999	0.996	0.999
LOD (µg/ml)	0.1065	0.2777	3.6580	0.8976
LOQ(µg/ml)	0.3227	0.8417	11.084	2.7210
Precision(%RSD)				
Interday	0.0857-1.4682	0.2525-1.6080	0.0022-0.2293	0.0221-0.1921
Intraday	0.1080-1.7118	0.2599-1.3906	0.0021-0.2712	0.0054-0.2342
Accuracy %Bias	0.0258-0.5372	0.0263-0.3520	0.0423-0.1038	0.0166-0.0617

Table 2: Summary of estimation of MOTO and AM in different brands

S.No.	Brand	Labeled amount (mg)	Amount found[a] (mg)	% of Labeled amount*	RSD
1.	AMTAS M	25 (MOTO)	24.90±0.027	99.60±0.581	0.590
		5 (AM)	4.98±0.169	99.80±0.341	0.336
2.	Amlopress-AT	25 (ATE)	24.87±0.033	99.58±0.563	0.541
		5 (AM)	4.96±0.176	99.71±0.473	0.308

a: data represents mean ± SD; n=3

Fig. 1: Overlain spectra of synthetic mixture of Metoprolol and Amlodipine besylate between 210-310 nm

REFFRENCEES

1. Tripathi KD. Essential of Medical Pharmacology, 5th Ed., Jaypee Brothers Medical Publishers (P) Ltd., 2004.
2. United States Pharmacopoeia, Asian edition, US pharmacopoeial convention, Inc.: US; 2003.
3. Indian Pharmacopoeia, Vol. II., Published by the Controller of Publication Delhi, 1996
4. Martindale. The Complete Drug Reference. 34th Ed. Pharmaceutical Press, USA; 2005.
5. European Pharmacopoeia, 6th Ed. V-2, Council of Europe, 2008.
6. Naidu KR, Kale UN and Shingare MS. Stability indicating RP-HPLC method for simultaneous estimation of Amlodipine besylate and Benzepril HCl from their combination drug products. J Pharm Biomed Anal. 2005;39(1-2):147-155
7. Agrekar AP and Powar SG. Simultaneous determination of Atenolol and Amlodipine besylate in tablets by HPTLC. J Pharm Biomed Anal. 2000;21(6):1137-1142.
8. International Conference on Harmonisation Draft Guideline on validatoion of Analtical Procedures: 1995. Defination and Terminology, Federal Register, 60, 11260.
9. Topale PR. Simultaneous UV Spectrophotometric estimation of Amlodipine besylate and Losartan Potassium in tablet dosage forms. Indian drugs. 40;119-121
10. Johannsson M. Determination of atenolol in plasma by dual-column liquid chromatography and Flurometric. J Chromattogr. 003;18(432):265-272
11. El-Gindy. HPLC and chemo metric-assisted spectrophotometric methods for simultaneous estimation determination of Atenolol, Amiloride hydrochloride and Chlorthalidone, Farmaco. 2005;60(3):269-278.