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SIMULTANEOUS QUANTITATION OF AMLODIPINE AND METOPROLOL IN COMBINATION BY UV SPECTROPHOTOMETRY

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ABSTRACT

The present work describes absorption ratio and absorption correction methods for simultaneous quantitative analysis of Amoldipine besylate and Metoprolol tartrate in the mixture using 0.1 N HCl. Overlain UV spectra of Amoldipine besylate and Metoprolol tartrate showed Isobestic wavelength at 257.4 nm and Metorpolol did not show any absorbance in region of 300 -400 nm. In absorption ratio method, the wavelengths for measurements used were 274 nm and 257.4 nm where as in absorption correction method Amoldipine besylate was estimated at 365 nm using standard absorptivity value and its absorbance had been corrected at 274 nm to quantify Metoprolol tartrate. The percent assay was found to be in the range of 97 - 106 for Amoldipine besylate in method I and II and 99 - 115 Metoproplol tartrate in method I and 93 – 110 for Metoprolol tartrate in method II. Both methods were validated in accordance with ICH guidelines for accuracy, precision, linearity, range, LOD and LOQ. The percent recoveries were found to be in the range 97.12 to 105.77 for Amoldipine and 99 to 115 for Metoprolol in method I, 97.12 – 105.72 for Amoldipine and 93.12 – 110.21 for Metoprolol in method II. The sample solution was found to be stable for about 12 hours during precision study.

Keywords: Absorption Ratio, Absorption Correction Method, Amlodipine Besylate and Metoprolol Tartrate

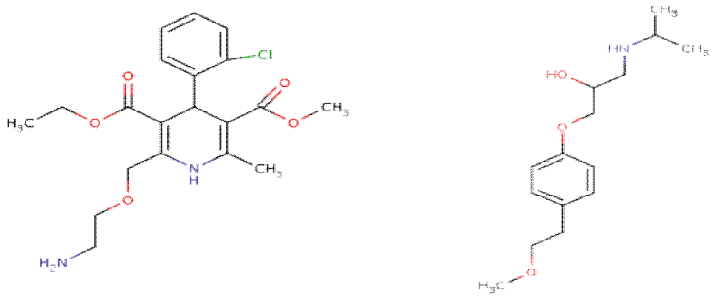
INTRODUCTION

Amlodipine (AMD), a dihydropyridine calcium channel blocker, inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure. Chemically it is 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate and used in the treatment of hypertension and angina **[1]**.

Metoprolol (MTP), a beta$_1$-selective drug which belongs to the chemical class of beta blockers, chemically known as {2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl}(propan-2-yl)amine is used in the treatment of hypertension and various cardiovascular disorders **[1]**.

Literature survey reveals that various UV methods like absorption ratio **[2, 3]**, Simultaneous equation **[3, 7]**, multi component **[4]**, derivative Spectroscopy **[5]** and absorption corrected **[2]** method have been reported for Simultaneous estimation of Metoprolol and Amlodipine in physical mixture and in tablet formulation using solvents like methanol, 0.1 N NaOH, distilled water.

Few HPLC methods were also found to be reported for simultaneous estimation Amlodipine, Metoprolol in combination **[6, 9, 10]**. The objective of the present work is to establish analytical method for simultaneous estimation of Metoprolol and Amlodipine in 0.1N HCl.



A) **B)**

Figure 1: A) Structure of Amlodipine and B) Structure of Metoprolol

MATERIALS AND METHODS

Amlodipine Besylate and Metoprolol Tartarate were obtained as gift samples from Micro lab Ltd Bangalore, India. All chemicals and reagents used were of Analytical Grade provided by Al Ameen College of Pharmacy, Bangalore.

Instrument

UV -1700, PHARMA SPEC (Shimadzu) UV/Visible spectrophotometer along with UV probe software was used for absorbance measurement, process and store the data. Acculab balance and Mark ultra sonicator were also used during the analysis.

Standard Stock Solution

Accurately 25 mg of AMD and MTP was weighed and transferred into 100 ml and 10 ml volumetric flasks respectively, dissolved in few ml of 0.1 N HCl by sonicated for 5 min and volume was made up with 0.1 N HCl.

Preparation of Calibration Curve

Appropriate aliquots were transferred from each standard stock solution into a series of 10 ml volumetric flasks. The volume was made up to mark with 0.1N HCl to get sets of solutions having concentration range 10 – 100 µg/ml and 50-300 µg/ml for AMD and 50-300 µg/ml, 100-600 µg/ml for MTP. These solutions were scanned in the UV region of 190 – 400 nm at fast mode and stored in UV probe software. The calibration curve was prepared by plotting the absorbance *vs* concentration at various wavelengths like 257.4 nm, 274 nm and 365nm.

Analysis of Mixture

Accurately 25 mg of AMD and 250 mg of MTP were weighed and mixed thoroughly. The sample solution was prepared by transferring 27.5 mg of the mixture into 100 ml volumetric flask, dissolved in few ml of 0.1 N HCl and sonicated for 5 min and volume was made up to mark with 0.1N HCl. The solution was filtered using whatmann filter paper 41 and accurately 5 ml of filtrate was transferred into 10 ml volumetric flask and the volume was made up with 0.1 N HCl. The sample solution was measured for absorbance at different wavelengths like 257.4 nm, 274 nm and 365nm and concentration of AMD and MTP were estimated using absorbance and equation mentioned in method I and II.

Method I: Absorption Ratio Method

The sample solution was measured at 257.4 nm and 274 nm and the absorption ratio for mixture QM was calculated. The concentration of MTP and AMD were determined using following equations where ax_1, ax_2, ay_1 and ay_2

$$\text{Conc of MTP} = \frac{Q_M - Qx}{Q_Y - Q_X} \times \frac{A_1}{a_{Y1}} \quad \text{--------- 1}$$

$$\text{Conc of AMD} = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{a_{x1}} \quad \text{---------2}$$

Q_M= ratio of absorbance of Mixture at 274 nm to absorbance of mixture at 257.4 nm.

Q_Y = ratio of absorbtivity of MTP at 274 nm to 257.4 nm

Q_x = ratio of absorbtivity of AMD at 274 nm to 257.4 nm

A_1= absorbance of mixture at 257.4 nm.

a_{X1}= Absortivity of AMD at 257.4 nm

a_{Y1}= Absortivity of MTP at 257.4 nm.

Method II: Absorbance Correction Method

From the overlain spectra AMD and MTP was studied in 0.1 N HCl, it was evident that AMD was absorbing in 365 nm and MTP showed zero absorbance and concentration of Amlodipine was calculated using 114 as standard absortivity value (A1%, 1cm) while concentration of MTP was calculated after correction of absorbance of AMP at 274 nm **(Figure 2)**.

$$\text{Conc}_{AMD} = \frac{A_1}{ax_1} \quad \text{……3}$$

$$\text{Conc}_{MTP} = \frac{A_2 - ax_2 C_{AMD}}{ay_2} \quad \text{……4}$$

A_1 and A_2 absorbtivity at 365 nm and 274 nm respectively.

Validation Parameters

Both the methods were validated as per ICH guidelines Q2 (R1) for accuracy, precision, linearity, range, LOD and LOQ.

Accuracy

Accuracy was confirmed by recovery study as per ICH norms at three different concentration levels 50%, 100% and 150%. The standard drug solutions were added to pre analyzed solution and then percentage of drug content was calculated. The result of accuracy study was reported in **Table 2**. From the recovery study it is clear that the method is accurate for quantitative estimation of MTP and AMD

Precision

The precision of the methods was determined by measuring %RSD of the results of sample and standard solutions obtained at three different time intervals like 0, 3, 6 hours for intraday study while the same solutions were analyzed at three consecutive days for inter day studies. Reproducibility study was performed by estimating the same sample of physical mixture by two different analysts.

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

The LOD and LOQ of AMD and MTP by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 s/S and 10 s/S respectively, where S is the mean slope of the calibration curve and s is the standard deviation of intercept.

RESULT AND DISCUSSION

Overlain UV spectra of MTP and AMD were studied in 0.1 N HCl and AMD was found to be absorbing in the entire range of 190-400 nm where MTP was found to absorbing in the 190 – 300 nm (as shown in **Figure 2**). Two isobestic wavelengths like 257.4 nm and 234.8 nm were observed where absorbance at 257.4 nm was found to be optimum for absorption ratio method.

In the absorption ratio method, two wavelengths like 257.4 nm and 274 nm were selected. The absorbtivity of each drug were determined from calibration curve on both wavelengths. It was found to be 136 (ax^1), 30 (ax^2), 13.33 (ay^1) and 38.66 (ay^2) where x is AMD and y is MTP and λ^1 and λ^2 are 257.4 nm and 274 nm respectively. The drug content was determined using absorption ratio of mixture (QM) and absorptivity ratio of individual drug (Q^x, Q^y) as discussed under method I using **Equation 1 and 2**.

MTP did not show any absorption in the region of 300 – 400 nm while AMD was found to be prominently absorbing. Hence absorbance correction method was applied to estimation of AMD and MTP simultaneously. AMD was quantified at 365 nm in 0.1 N HCl using 114 as standard absorptivity value (A 1%, 1 cm) at about 365 nm (where MTP was zero). MTP was evaluated after correcting the absorbance of AMD at 274 nm using the equation 4 as discussed under method II.

Marketed formulation like Metolor AM was analysed using these methods. The % RSD for assay was found to be less than 3% indicating the precision of the method. The assay results for formulation were found to be high and outside the official limits. This may be due to interference of polymers used for controlled release while assay was found to accurate and reproducible for the physical mixture.

The mean percentage assay was found to be 101.44 for AMD and 107.09 for MTP in method I and 101.42 for AMD and 101.66 for MTP in method II respectively.

Method I and II, both were validated as per ICH guidelines. The accuracy was determined by standard addition method (50%, 100% and 150%) and the percent recoveries were found to be in the range 97.12 to 105.77 for AMP and 99.70 to 114.49 for MTP in method I, 97.12 to 105.72 for AMD and 93.12 to 110.21 for MTP in method II.

Precision was determined by intra, inter day and reproducibility studies % RSD was found to be less than 3% except for inter day study. The standard and sample solutions were found to be stable for about 24 hours, hence % RSD was found to be more than 5% for intraday.

The linearity and range were determined from calibration curve AMD was found to be linear in the concentration range of 10 – 60 µg/ml at 257.4 nm, 50-300 µg/ml at 274 nm and 10 – 100 µg/ml at 365 nm with co efficient correlation 0.999. While MTP was linear in the range of 100 – 600 µg/ml at 257.4 nm and 50-300 µg/ml at 274 nm with co efficient correlation 0.999.

LOD and LOQ were determined using mean slope and standard deviation of intercept of C. LOD was found to be 0.445 µg/ml at 257.4 nm, 4.657µg/ml at 274 nm and 0.655 µg/ml at 365 nm for AMD and 15.29 µg/ml at 257.4 nm, and 20.85 µg/ml at 274 nm for MTP respectively.

LOQ was found to be 1.484 µg/ml at 257.4 nm, 7.532 µg/ml at 274 nm and 2.185 µg/ml at 365 nm for AMD and 50.98 µg/ml at 257.4 nm and 69.52 µg/ml at 274 nm for MTP respectively.

Hence these methods are suitable for simultaneous estimation of AMD and MTP in physical mixture and in conventional combination tablets with high accuracy and precision.

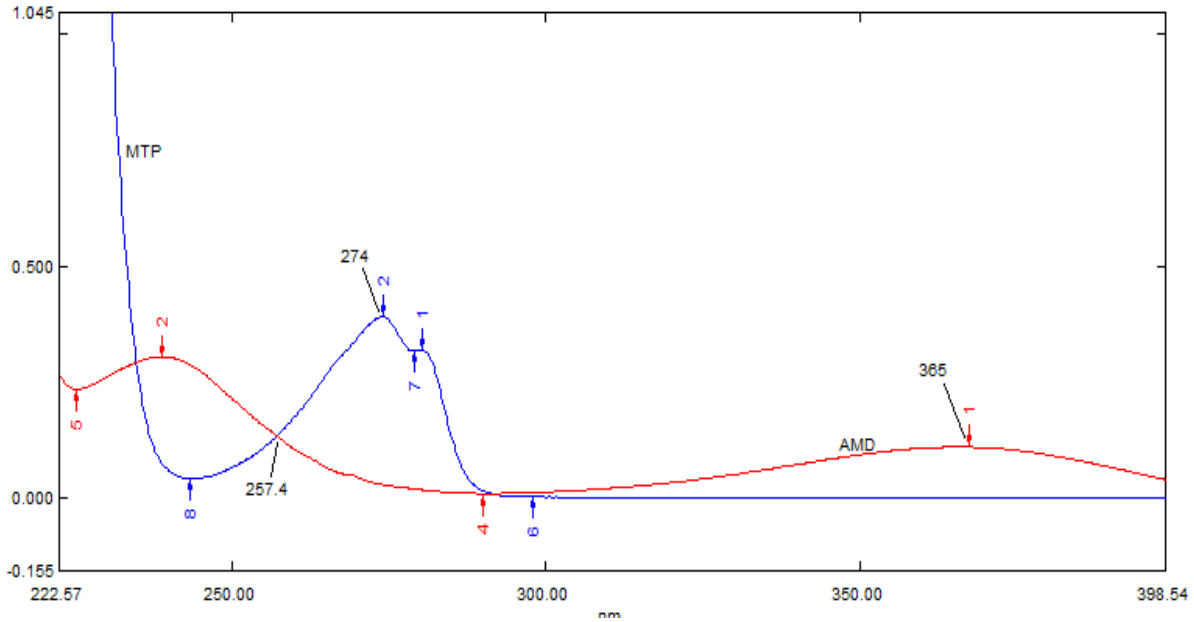


Figure 2: Overlaying Spectra of 10 mcg/ml of Amoldipine and 100 mcg/ml of Metoprolol

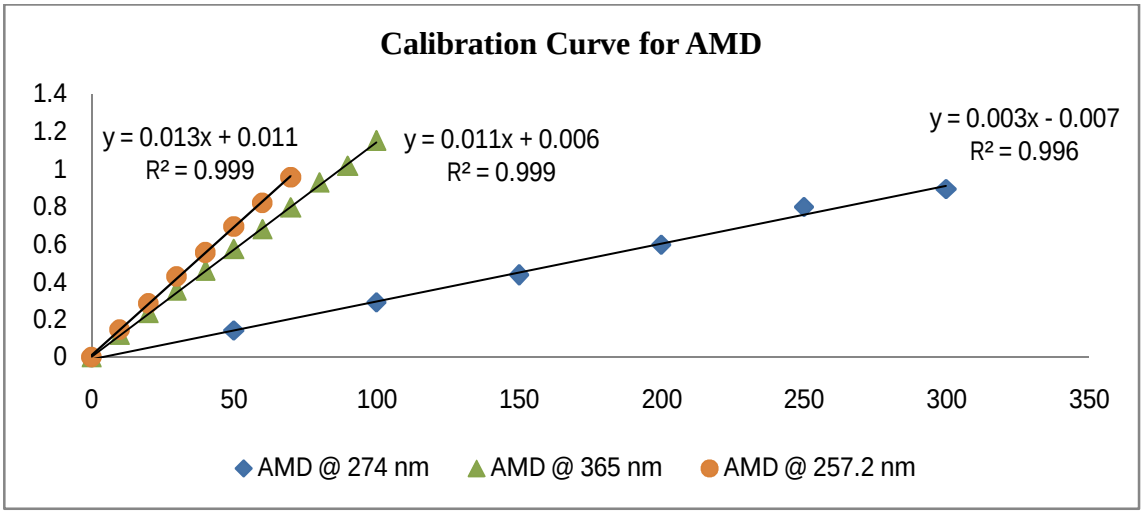


Figure 3: Calibration Curve for Amlodipine

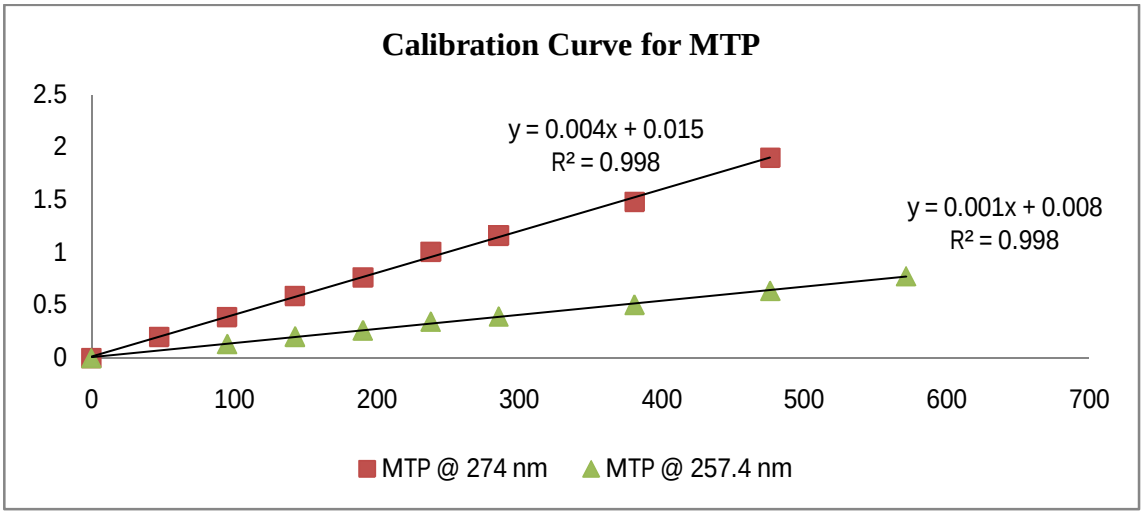


Figure 4: Calibration curve for Metoprolol

Table 1: Summary for Linearity and Range Studies

Analytes	AMD			MTP	
Wave length	257.4 nm	274 nm	365 nm	257.4 nm	274 nm
Range (µg/ml)	10 – 60	50-300	10 – 100	100 – 600	50-300
Slope	0.0136	0.0031	0.0114	0.0013	0.004
Intercept	0.0155	0.0076	0.0062	0.008	0.0154
Correlation co efficient	0.9995	0.9964	0.9995	0.9987	0.9985
A(1%, 1cm)	136	30	114	13.33	38.66
LOD (µg/ml)	0.445	4.657	0.655	15.29	20.85
LOQ (µg/ml)	1.484	7.532	2.185	50.98	69.52

Table 2: Results for Assay, Precision and Accuracy

Parameters	Method I		Method II	
	AMD	MTP	AMD	MTP
Assay (%)(n=3)	101.445	107.095	101.42	101.665
Precision (% RSD)				
Intra day	<3%	<3%	<3%	<3%
Inter day	>5%	<3%	>5%	<3%
Reproducibility	<3%	<3%	<3%	<3%
Recovery (%)	103.47	99.70	103.29	93.12
50% Addition	105.77	104.95	105.72	101.21
100% Addition	97.12	114.49	97.12	110.21
150% Addition				

CONCLUSION

Absorbance ratio and absorbance correction methods have been developed and validated for simultaneous estimation of Metoprolol and Amlodipine. The proposed methods were found to be accurate and precise and can be used for routine analysis of Amlodipine and Metoprolol combined conventional tablets

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