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Original Article

ABSORPTION CORRECTION METHOD FOR SIMULTANEOUS ESTIMATION OF MOXONIDINE AND AMLODIPINE BESYLATE IN COMBINED PHARMACEUTICAL FORMULATION

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ABSTRACT

Hypertension is the most common disorder found in people nowadays. Combined therapy with Moxonidine and Amlodipine has high antihypertensive efficiency as well as decrease insulin resistance in hypertensive patients with metabolic syndrome. This had prompted many investigators to develop methods for simultaneous estimation of Moxonidine and Amlodipine Besylate in combined Pharmaceutical formulation.

A UV-Spectrophotometric absorption correction method has been developed for simultaneous determination of Moxonidine and Amlodipine Besylate in combined pharmaceutical formulation. Absorption correction method was based on the property of additivity of absorbances. Beer's law was obeyed concentration range of 5-25 µg/ml for Amlodipine Besylate and 3-15 µg/ml for Moxonidine.

This method was found to be simple, sensitive, accurate, precise, reproducible and economical and can be applicable for the simultaneous determination of Amlodipine Besylate and Moxonidine in combined dosage form(Moxovas-A).

The method offers the advantages of rapidity, simplicity and sensitivity and low cost and can be easily applied without the need for expensive instrumentation and reagents.

Keywords: Amlodipine Besylate, Moxonidine, Absorption correction method, Validation, Combined pharmaceutical formulation.

INTRODUCTION

Moxonidine is chemically (4-Chloro-*N*-(imidazolidin-2-ylidene)-6-methoxy-2-methylpyrimidin-5-amine).Moxonidine is used for the treatment of mild to moderate essential hypertension[1-3]. It is a selective agonist at the imidazoline receptor subtype 1 (I_1).It may be used alone or in combination with other antihypertensive drugs. It is official in British Pharmacopoeia (BP)[2]. BP describes HPLC method for its estimation. Various methods like HPTLC [4], RP-HPLC [5] and GC [6] for determination of MOX are reported in literature for estimation of MOX in pharmaceutical formulation.

Amlodipine Besylate (AML BES) is chemically (3-ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate). Amlodipine besylate is calcium channel blocker [2, 7, 8].It is used to treat diseases like hypertension, chronic stable angina and vasospastic angina. Amlodipine Besylateis official in British Pharmacopoeia (BP)[2]and Indian Pharmacopoeia (IP)[8]. IP and BP describe HPLC method for its estimation. Various methods like spectrophotometric [9,10] and HPLC [11-13]method for simultaneous estimation of AML BES with other drug, are reported in literature for estimation of AML BES in combined pharmaceutical formulation. The combined dosage forms of MOX and AML BES are available in the market for the treatment of hypertension.

The present manuscript describes alternative simple, sensitive, accurate, precise, reproducible, and economical absorbance correction method for simultaneous estimation of AML BES and MOX in combined pharmaceutical formulation.

MATERIAL AND METHODS

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).AML BES and MOX bulk powder was obtained by West Coast pharmaceuticals Pvt. Ltd., Ahmedabad, GUJ, India and Macleods Pharmaceuticals Pvt.Ltd. respectively. The commercial fixed dose combination Moxovas-A tablet was procured from the local market. All other chemicals used were of analytical grade. Calibrated glasswares were employed throughout the work.

Preparation of standard stock solution

Moxonidine standard stock solution (100 µg/ml): Accurately weighed 10 mg of Moxonidinewas taken in 100 ml volumetric flask and diluted with Methanol up to the mark.

Amlodipine Besylate standard stock solution (100 µg/ml): Accurately weighed 10 mg of Amlodipine Besylate was taken in 100 ml volumetric flask and diluted with methanol up to the mark.

Selection of analytical wavelength

By appropriate dilution of two standard drug solutions with methanol, solutions containing 9µg/ml of Moxonidine and 15µg/ml of Amlodipine Besylate were scanned separately in the range of 200-400 nm. Overlain spectra show 253 nm as the λ max of MOX and 360 nm as the λmax of AML BES.

Method (Absorption correction method)

From the overlay spectra [Fig 3] of AML BES and MOX, two wavelengths were selected, one at 253nm (λ2) for MOX and the other at 360nm (λ1) for AML BES at which MOX shows zero absorbance. The absorbances of the sample solutionswere measured for both the drugs at selected wavelengths. The concentrations of drugs in sample solution were determined by using the following formula:

$$Cx = \frac{A_1}{ax_1} \qquad C_y = \frac{A_2 - (C_x\, ax_2)}{ay_2}$$

Where,

C_x = Conc of Amlodipine Besylate

C_y= Conc. Of Moxonidine

A_1 = Absorbance of Sample Solution at λ1 (360 nm)

A_2=Absorbance of Sample Solution at λ2 (253 nm)

ax_2, ay_2 = Absorptivities of AML BES and MOX at λ2 (253 nm)

ax_1 = Absorptivityof AML BES λ1 (360nm)

Analysis of pharmaceutical formulation

Twenty tablets were weighed and crushed to powder. The quantity of the powder equivalent to 10 mg of AML BES and 0.4 mg of MOX was transferred to a 100 ml volumetric flask. To it 4.6 mg of std API of MOX was added to make the ratio 2:1. The content was mixed with Methanol (70 ml) and sonicated for 20 min to dissolve the drug as completely as possible. The solution was then filtered through a Whatman filter paper no. 41. The volume was adjusted up to mark with methanol. [MOX (50µg/ml) & AML BES (100 µg/ml)].

An aliquot of this solution (1.0 ml) was transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol to make final concentration of Amlodipine Besylate (10 µg/ml) and Moxonidine (5µg/ml).[Table 2].

Method Validation

Method validation was performed following ICH guidelines [14]. The proposed method has been extensively validated in terms of linearity, accuracy and precision, limit of detection and limit of quantification.

Linearity (Calibration curve)

Calibration curves[Fig 7-9] were plotted over a wide concentration range and the linear response was observed over a concentration range of 3-15µg/ml for Moxonidine and 5-25µg/ml for Amlodipine Besylate. Accurately measured standard solutions of Moxonidine(0.3, 0.6, 0.9, 1.2, 1.5ml) and Amlodipine Besylate (0.5, 1.0, 1.5, 2.0, 2.5ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with Methanol, and the absorbance was measured(n=3) at 253 nm (λmax of Moxonidine) and at 253 nm and 360 nm (λmax of Amlodipine Besylate). The calibration curves were constructed by plotting absorbance vs. concentrations. The linear regression equation of Moxonidine was y = 0.0323x + 0.0262 (R^2= 0.9965) and Amlodipine Besylate at 253 nm was y = 0.0139x – 0.0497 (R^2= 0.9992) and at 360nm was y = 0.011x + 0.0091(R^2= 0.9998).

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the prequantified sample preparation at three different concentration levels 80 %, 100 % and 120 %, taking in to consideration percentage purity of added drug sample. The amounts of Amlodipine Besylate and Moxonidine were estimated by applying obtained values to the respective equations. Each concentration was analysed 3 times and average recovery were measured.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter-day and reproducibility.

The repeatability was evaluated by assaying 6 times of sample solution of 9 µg/ml Moxonidine and 15 µg/ml Amlodipine Besylate determination without changing the parameter. The intra-day and inter-day precision study of Moxonidine and Amlodipine Besylate was carried out by estimating different concentration of Moxonidine (6, 9, 12µg/ml) and Amlodipine Besylate (10, 15, 20µg/ml), 3 times on same day and on 3 different day (first, second and third).

Limit of detection and limit of quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 × (SD/Slope) and 10 × (SD/Slope) criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slope of the calibration curve.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

S. No.	Validation Parameter	Amlodipine Besylate (360 nm)	Moxonidine (253 nm)
1	Linearity		
	Regression Equation	Y=0.011x + 0.0091	Y=0.0323x + 0.0262
	Regression Coefficient	0.9998	0.9965
2	Range	5-25	3-15
3	Accuracy(%Recovery)	98.45-101.30	98.36-101.66
4	Precision (%RSD)		
	Repeatability	1.388	1.722
	Intraday	1.275-1.424	0.766-1.326
	Inter-day	1.46 – 1.96	1.263 – 1.873
5	LOD (µg /ml)	0.175	0.097
6	LOQ (µg /ml)	0.530	0.2969

Table 2: Analysis of Tablet (n = 3)

Active Ingredent	Label claim	Test Concentration (µg/ml)	% Assay
Moxonidine	0.2 mg	5	100.16±1.342
Amlodipine Besylate	5 mg	10	100.17±1.117

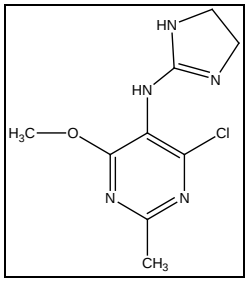

Fig. 1: Structure of Moxonidine

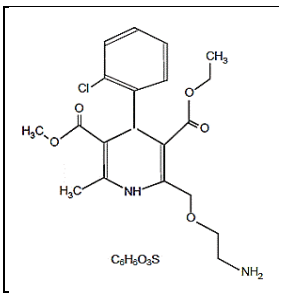

Fig. 2: Structure of Amlodipine Besylate

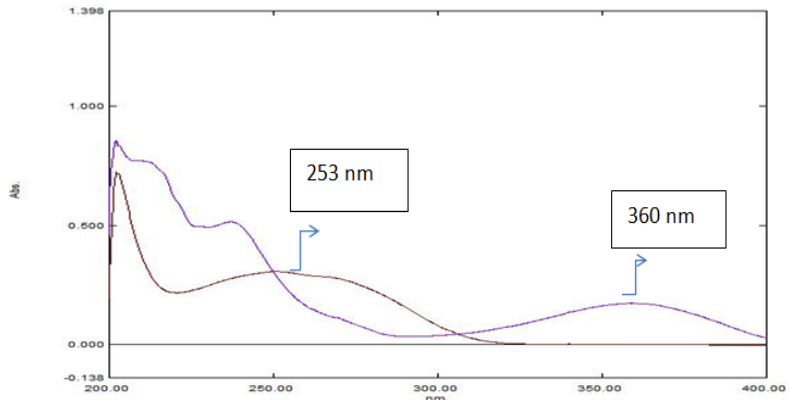

Fig. 3: UV Spectrum for Moxonidine (9 µg/ml) and Amlodipine Besylate(15 µg/ml).

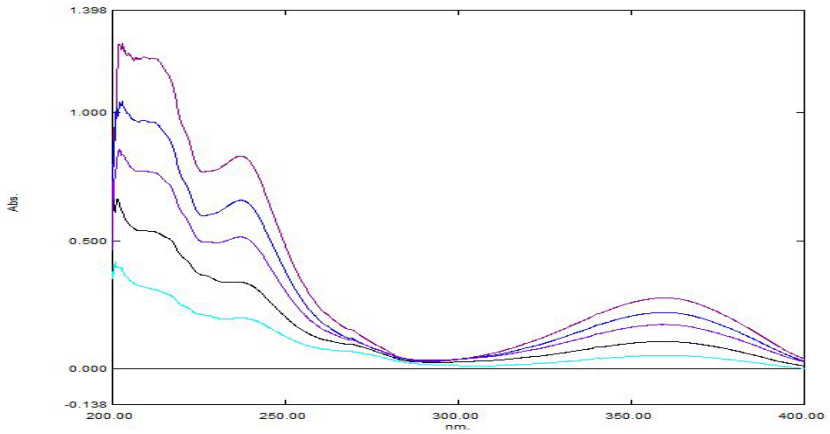

Fig. 4: Overlain spectrum of Amlodipine Besylate(5-25µg/ml)

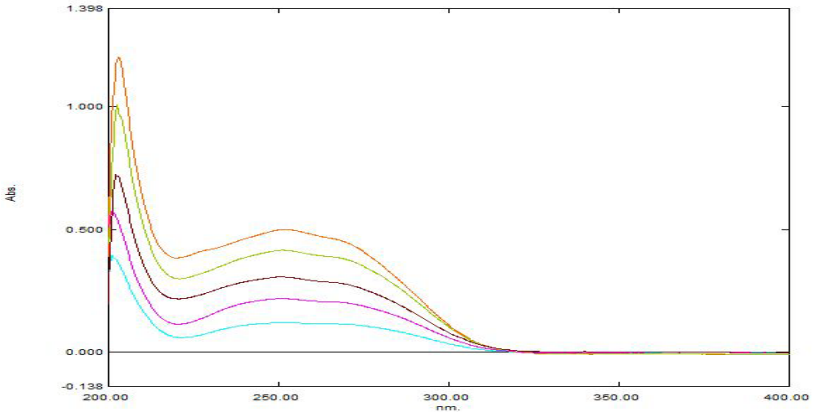

Fig. 5: Overlain spectrum of Moxonidine (3-15µg/ml).

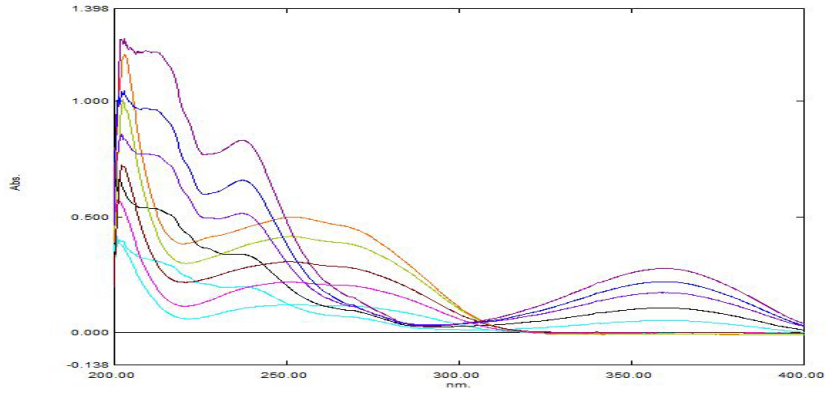

Fig. 6: Overlain spectrum of Amlodipine Besylate(5-25µg/ml) and Amlodipine Besylate (3-15µg/ml)

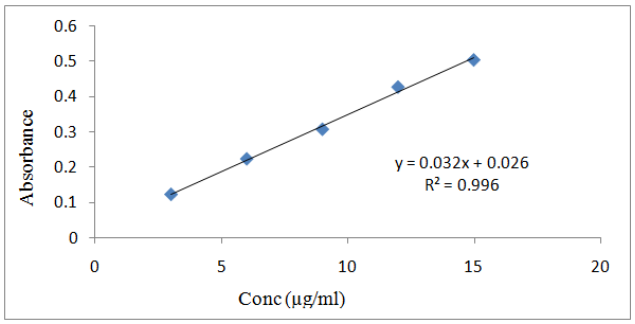

Fig. 7: Calibration curve of Moxonidine at 253 nm

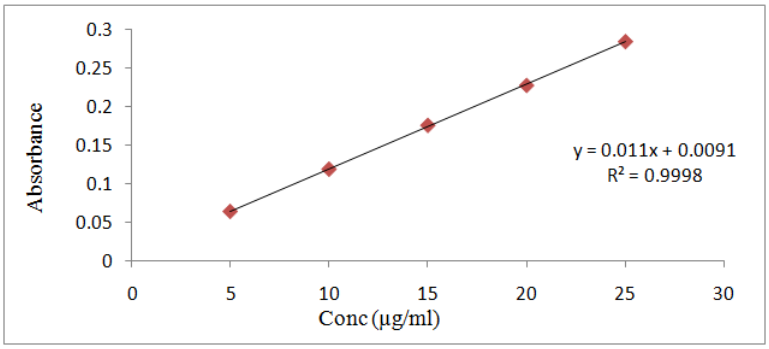

Fig. 8: Calibration curve of Amlodipine Besylate at 360 nm

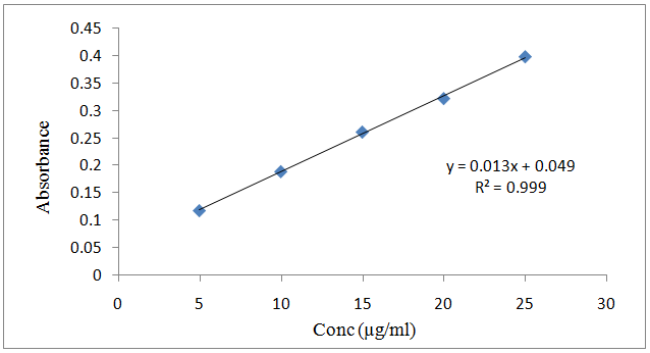

Fig. 9: Calibration curve of Amlodipine Besylate at 253 nm

RESULT AND DISCUSSION

A reliable absorption correction method was developed for simultaneous estimation of Moxonidine and Amlodipine Besylate in combined pharmaceutical formulation by UV Spectrophotometry. Beers law was obeyed in concentration range of 3-15µg/ml for Moxonidine and 5-25µg/ml for Amlodipine Besylate at 253 nm and 360 nm wavelengths, respectively. The correlation coefficient Moxonidine and Amlodipine Besylate was found to be R^2= 0.996 and 0.999. The mean % recoveries were found to be in the range of 98.45-101.30% and 98.36-101.66% for Amlodipine Besylate and Moxonidine respectively. The LOD and LOQ were 0.175µg/ml and 0.530µg/ml of Amlodipine Besylate and 0.097µg/ml and 0.2969µg/ml of Moxonidine, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analytes, which can be applied for the analysis of Amlodipine Besylate and Moxonidine in combined pharmaceutical formulation [Table 1].

CONCLUSION

The results of our study indicate that the proposed UV spectroscopic method is simple, rapid, precise and accurate. The developed UV spectroscopic method was found suitable for determination of Moxonidine and Amlodipine Besylate in combined pharmaceutical formulation without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of Amlodipine Besylate andMoxonidine. It can therefore be conclude that use of the method can save time and money and it can be used in small laboratories with accurate and wide linear range.

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