VALIDATED U.V. SPECTROPHOTOMETRIC METHOD OF SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND TELMISARTAN IN BULK AND IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, rapid and precise simultaneous analytical method has been developed for simultaneous determination of Amlodipine Besylate and Telmisartan in bulk and in their combined tablet dosage form. Simulated gastric fluid of pH 1.2 was used as medium. Absorption Ration method was developed and is validated statistically as per ICH guidelines. The absorptions were observed at 291nm and 324nm which were selected based on overlap spectra of Amlodipine Besylate and Telmisartan. Linearity was observed by linear regression equation method for both drugs in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The % R.S.D. were found to be less than 2 % as required by ICH guidelines, which indicates the validity of methods. Statistical analysis proves that the method is reliable, sensitive, reproducible and selective for the simultaneous estimation of Amlodipine Besylate and Telmisartan.

KEYWORDS: Absoption Ratio method, Amlodipine Besylate, Telmisartan, Simulated Gastric Fluid, etc.

INTRODUCTION

Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker and chemically known as 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By

inhibiting the influx of calcium in smooth muscle cells, Amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. The molecular formula is $C_{20}H_{25}ClN_2O_5$ and the structural formula is in figure 1. It has a molecular weight of 408.879 g/mol.[1-3] Telmisartan chemically is 2-(4-{[4-Methyl-6-(1- methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3- benzodiazol-1-yl]methyl}phenyl)benzoic acid. It is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. The molecular formula is $C_{33}H_{30}N_4O_2$ and the structural formula is in figure 2. It has a molecular weight of 514.617 g/mol.[4-6]

In the literature survey, some methods are reported in combination for estimation of Amlodipine Besylate and Telmisartan by HPTLC[7] and some methods reported in combination for the estimation in pharmaceutical dosage form in methanol by UV Spectrophotometry.[8,9] Extensive literature survey reveals that no Spectrophotometric method is available for simultaneous determination of Amlodipine Besylate and Telmisartan in combination in physiologic body fluids. Aim of present work was to develop simple, precise, accurate and economical Spectrophotometric methods for simultaneous determination of binary drug formulation in biological fluids at three different pH. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines.[10]
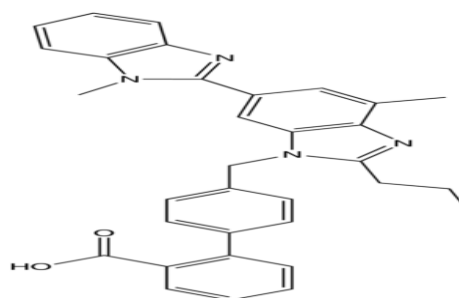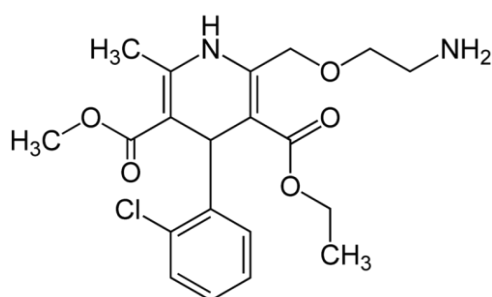

Fig. 1 Structural formula of Amlodipine Besylate   Fig. 2 Structural Formula of Telmisartan

MATERIALS AND METHODS

Amlodipine Besylate (AB) and Telmisartan (TEL) obtained from USV Ltd, Baddi. A commercial sample AB and TEL tablets were procured from local market and used within

their shelf-life period. Potassium Chloride and Hydrochloric acid was procured from Loba Chemie Pvt. Ltd., Mumbai and S.D. Fine Chemical Limited, India respectively. Quantitative estimation was performed on Shimadzu UV 1700 double beam UV-Visible spectrophotometer with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a medium scan speed, setting slit width to be 1 nm and sampling interval to be auto. pH meter (Labindia) was used.

The absorption ratio method

The absorption ratio method is a modification of the simultaneous equation procedure. It depends on the property of the substance such that it obeys beers law at all wavelength, the ratio absorbance at any two wavelengths is a constant value independent of concentration or pathlenght e.g. Two dilutions having the same absorbance ratio A1/A2. In USP, this ratio is referred as *Q value*. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances are measured at two wavelengths. One being the λ_{max} of one of the components and the other being a wavelength of equal absoptivity of the two components i.e. isoabsorptive point.[11-12]

Preparation of Simulated gastric fluid (SGF)

50ml of 0.2M KCl was taken in 200ml volumetric flask. 85ml of 0.2M HCl was added to the volumetric flask. This solution was diluted to 200ml with distilled water.

Experimental condition

According to the solubility characteristics, the drugs were first dissolved in methanol and further dilutions were carried out with SGF (pH 1.2).

Preparation of Standard Stock solution

Accurately weighed quantity of AB (14 mg approx) (35.67 mg of Amlodipine Besylate is equivalent to 25.62 mg of Amlodipine) and TEL (10mg) was transferred to two separate 100 mL volumetric flask, dissolved in 10 mL methanol and diluted to the mark with SGF of pH 1.2 to get final concentration 100 μg/ml of each drug.

Spectral Analysis and Selection of wavelengths

The aliquots of standard stock solution of drugs were diluted separately with SGF of pH 1.2 to obtain final concentration of 10 μg/ml of each drug. Each working standard solution was scanned between 200-400 nm in Shimadzu UV visible spectrophotometer. Overlain

absorption spectrum of both drugs was recorded and is depicted in Fig. 3. Two wavelengths were selected. Among the two, 291 nm is a λ$_{max}$ of TEL and 324 nm is an isobestic point. Then the absorbance was measured at 291nm and 324nm and calculated the absorptivity.

Preparation of sample solution

Twenty tablets (marketed) were weighed and their mean weight was determined. The tablets were triturated to a fine powder. For the analysis of AB, a standard addition method was used. An accurately weighed amount of pure AB was added to finely powdered sample to bring the concentration ratio of 1:1. The amount of powder equivalent to 50 mg of AB and 50 mg of TEL were weighed and transferred into 100 ml volumetric flask. Methanol (10 mL) was added to it and sonicated for 10 minutes and volume was made up to mark with SGF. The solution was filtered through Whatmann filter paper No. 41. From the above solution suitable aliquot was diluted with SGF to get concentration 10 μg/mL of each drug. The absorbance of sample solutions were measured at both selected wavelengths.
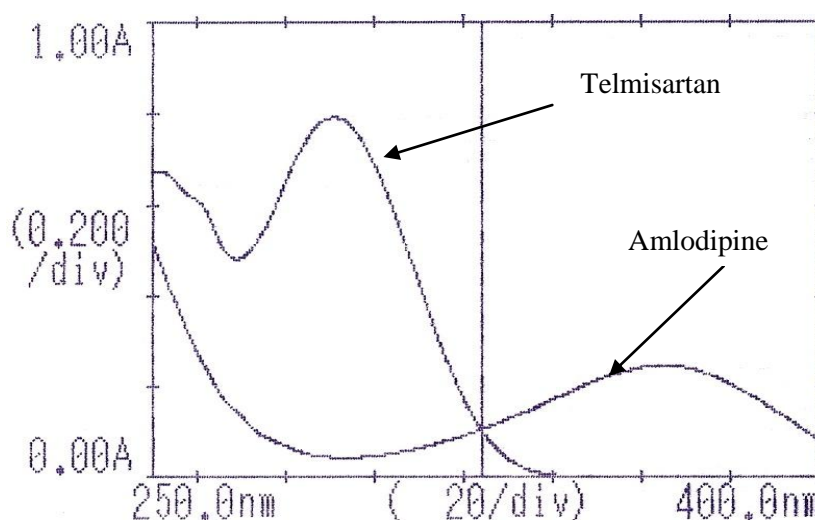

Fig. 3 Overlain Absorption Spectra of Amlodipine Besylate and Telmisartan in SGF of pH 1.2

Validation of proposed method[10]

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision, accuracy, LOD and LOQ for the analyte.

• **Linearity:** It was determined by measuring the absorbance of various concentrations of both drugs Amlodipine Besylate and Telmisartan at the λ$_{max}$ of Telmisartan i.e. 291 nm and at

isoabsorptive point i.e. 324 nm. The response was plotted against concentration of the analyte. Linearity of the calibration curve was demonstrated by applying least square regression analysis to the plot obtained.

• **Accuracy:** To determine accuracy of the proposed method, three different levels of drug concentrations were prepared from independent stock solution and analyzed. Accuracy was assessed as the mean percentage Bias.

• **Precision:** Intra-day and inter-day variation were taken to determine intermediate precision of the proposed method. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. The same drug concentrations were prepared on three different days to study inter-day variation. %Relative standard deviation (%RSD) were calculated which should be less than 2%.

• **Limit of detection (LOD) and Limit of quantitation (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. The standard deviation of the response can be obtained either by measuring the standard deviation of the blank response or by calculating the residual standard deviation of the regression line or by calculating the standard deviation of the y-intercept of the regression line, i.e. the standard error of the estimate. Similarly, LOQ was determined using the relation 10 σ/s.

RESULT AND DISCUSSIONS

An absorption ratio method procedure was proposed as a suitable method for the analysis of drugs AB and TEL in dosage forms. This method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other being the λ_{max} of one of the two compounds. From the stock solutions, working standard solutions of AB and TEL were prepared by appropriate dilution and were scanned in the entire UV range to determine the λ_{max} and isoabsorptive point. AB and TEL have λ_{max} at 238.4nm and 291nm, respectively. Both the drugs were found to have same absorbance at 324nm, which was taken into consideration as isoabsorptive point (Figure 2). A series of standard solutions were prepared for AB and TEL both was prepared and absorbances of solutions were recorded at 291nm and 324nm to plot a calibration curve of absorbance versus concentration. The calibration curves were found to be linear in concentration range under study. Regression equation and

Absorptivity values of AB and TEL were determined at selected wavelengths are presented in Table 1.

Table 1 Result of Analytical method Development in SGF

Parameter		Amlodipine at 291nm	Telmisartan at 291nm	Amlodipine at 324 nm	Telmisartan at 324 nm
Absorptivity		12.082	533.276	70.875	67.043
Regression equation	Slope	0.001	0.053	0.007	0.006
	Intercept	0.0238	0.0022	0.0098	0.0136
	Correlation coefficient(r^2)	0.9988	0.9988	0.9988	0.9988

The concentration of two drugs in mixture was calculated by using following equations.

$$C_T = \frac{(Q_M - Q_B)A_{MI}}{(Q_T - Q_A)a_{TI}} \qquad C_A = \frac{(Q_T - Q_M)A_{MI}}{(Q_T - Q_A)a_{TI}}$$

$$Q_M = \frac{A_{MT}}{A_{MI}} \qquad Q_A = \frac{a_{AT}}{a_{AI}} \qquad Q_T = \frac{a_{TT}}{a_{TI}}$$

Where C_T and C_A are the concentrations of TEL and AB. $\mathbf{A_{MI}}$ is the absorbance of mixture at isoabsorptive point (324 nm), $\mathbf{A_{MT}}$ is the absorbance of mixture at λ_{max} of TEL (291 nm), $\mathbf{a_{AI}}$ and $\mathbf{a_{TI}}$ are the absorptivities of AB and TEL respectively at 324 nm, $\mathbf{a_{AT}}$ and $\mathbf{a_{TT}}$ are absorptivities of Amlodipine Besylate and Telmisartan at 291 nm.

The percentage of purity of AB and TEL in tablet dosage form is shown in Table 2. The precision of the spectrophotometer system was determined using the %RSD of the absorbance for six replicate injections of the drug. The %RSD was less than 2 indicating precision of the method. Precision data were present in Table 2.

Table 2 Determination of AB and TEL in combined tablet dosage form

Brand	Tablet content	Label content (mg)	Amount found (mg)	%Amount*	± SD*	RSD (%)*
I	AB	5	4.96	99.20	± 0.045	0.384
	TEL	40	39.87	99.67	± 0.028	0.184
II	AB	5	4.95	99.00	± 0.053	0.485
	TEL	40	40.04	100.10	± 0.035	0.295

Mean of six readings

To prove the validity and applicability of the proposed method, studies were carried out as per ICH Guidelines and their results are stated in Table 3. Satisfactory results were obtained with % Bias and %RSD value less than 2%; thus conforming the accuracy and precision of proposed method. LOD and LOQ values for AB and TEL are stated in Table 3.

Table 3 Validation of proposed method

Parameter		Amlodipine at 291nm	Telmisartan at 291nm	Amlodipine at 324 nm	Telmisartan at 324 nm
Beer's Law Limit (µg/ml)		200-1000	4-23	30-170	30-180
LOD (µg/ml)		1.48	0.08	0.39	0.49
LOQ (µg/ml)		4.47	0.25	1.19	1.49
Precision (%RSD)	Interday	0.027-0.458	0.114-0.307	0.076-0.145	0.152-0.351
	Intraday	0.018-0.118	0.094-0.326	0.050-0.115	0.103-0.370
Accuracy (% Bias)		0.111-0.289	0.032-0.102	0.041-0.257	0.152-0.494

CONCLUSION

The proposed method for simultaneous estimation of Amlodipine Besylate and Telmisartan in combined dosage form was found to be was accurate, precise, sensitive and rapid. The advantages of proposed method were its simple procedure for sample preparation. Linearity was observed by linear regression equation method for both drugs in different concentration range in SGF of pH 1.2. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The % R.S.D. values were found to be less than 2 % as required by ICH guidelines, which indicates the validity of methods; thus confirmed the suitability of proposed method for the routine analysis of AB and TEL in pharmaceuticals.

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