

First Derivative Synchronous Spectrofluorimetric Quantification of Telmisartan/Amlodipine Besylate Combination in Tablets

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ABSTRACT: A first derivative synchronous spectrofluorimetric method has been developed and validated for simultaneous determination of telmisartan (TEL) and amlodipine besylate (AML) in combined tablet dosage form without any prior separation of components from the sample. TEL was determined at emission wavelength of 675 nm (zero-crossing wavelength point of AML). Similarly, AML was measured at 458 nm (zero-crossing wavelength point of TEL). The first derivative amplitude-concentration plots were rectilinear over the range of 4-14 µg/ml for TEL and 1-6 µg/ml for AML. The method was validated statistically as per ICH guidelines. Limit of detection (LOD) and quantification (LOQ) are reported. The % assay in commercial formulation was found to be in the range 99.60 – 100.22 and 98.40 – 99.80 for TEL and AML, respectively by the proposed method and % RSD values for precision and accuracy studies were found to be less than 2. The proposed method can be successfully applied for routine analysis of TEL and AML in tablets.

Key words: Amlodipine besylate, Telmisartan, First derivative, Synchronous, Spectrofluorimetry.

INTRODUCTION

Telmisartan (TEL) (Figure 1A) chemically known as 4'-[(1,4'-Dimethyl-2'-propyl[2,6'-bi-1*H*-benzimidazol]-1'yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid, is an angiotension-II (AT₁) receptor antagonist used in the treatment of hypertension and myocardial infarction.¹ TEL is an official drug in the British Pharmacopoeia (BP) which suggests a high performance liquid chromatographic (HPLC) procedure for the assay of the bulk powder.²

Amlodipine besylate (AML) (Figure 1B) chemically known as 3-ethyl-5-methyl 2-(2-amino ethoxy methyl)-4-(2-chloro phenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate benzene sulpho-nate, is a calcium channel blocker used in the treatment of hypertension and angina pectoris. It is

an official drug both in the Indian pharmacopoeia (IP) and British Pharmacopoeia (BP) which suggest a high performance liquid chromatographic method for the assay of bulk powder and its tablet formulation.³ TEL and AML have been formulated in a fixed-dose combination and used in the treatment of hypertension. Few analytical methods include spectrophotometry⁴ and high performance liquid chromatographic methods (HPLC)^{5,6} were developed for simultaneous quantification of TEL and AML in fixed-dose combination. To the best of our knowledge, no spectrofluorimetric method has been reported yet for quantification of TEL and AML in combined formulations, including tablet.

Spectrofluorimetric methods have been found more selective than normal UV-spectroscopy due to quantification of substance at excitation and emission wavelengths.^{7,8} Derivative spectrofluorimetry provides a greater selectivity and spectral

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discrimination than common spectrofluorimetry.^{9,11} It is the powerful approach for resolution of one analyte whose peak is hidden by a large overlapping peak of another analyte in multi-component analysis. Synchronous fluorescence spectroscopy (SFS) has been found to have several advantages such as simple spectra, high selectivity and low interference.^{12,13} The combination of synchronous scanning spectrofluorimetry with derivative techniques is advantageous in terms of sensitivity, spectral discrimination and more reliable identification of chemical species in multi component analysis.^{14,15} The aim of the present work was to develop a simple, economic, sensitive and rapid method for the simultaneous determination of TEL and AML in tablet dosage form by first derivative synchronous fluorimetry (FDSF) based on their native fluorescence. The emission spectra of TEL and AML were overlapped. It was difficult to analyze and determine their contents by conventional fluorimetry. These problems were minimized by using FDSF.

MATERIALS AND METHODS

Materials

All chemicals and reagents were of analytical grade. Telmisartan (TEL) and Amlodipine besylate (AML) were obtained as gift samples from Dr. Reddy's Laboratories Ltd., Hyderabad, India. Telsartan-AM and Sartel-AM formulations (TEL-40 mg and AML-5 mg) were purchased from the local pharmacies. Hydrochloric acid was procured from SD Fine Chemicals Ltd., Mumbai, India.

Apparatus. The fluorescence spectra and measurements were recorded using a Shimadzu (Japan) RF-5301 PC spectrofluorophotometer, equipped with 150 watt Xenon arc lamp, 1cm quartz cell, connected to RFPC software. The instrument was operated both at low and high sensitivity with excitation and emission slit width set at 5 nm. The drugs and reagents were weighed on an analytical balance (Shimadzu AUX 220, Japan).

Preparation of standard solutions. Standard stock solutions 100 µg/ml for TEL and AML was

prepared in 100 ml of 1 molar (M) hydrochloric acid (HCl) as solvent. Working solutions were prepared separately by making serial dilutions from the standard solution to obtain concentration between 4-14 and 1-6 µg/ml for TEL and AML, respectively and fluorescence intensity was quantified by spectrofluorimeter.

Procedure for sample preparation. Twenty tablets of each marketed formulation (Telsartan-AM and Sartel-AM), each containing 40 mg of TEL and 5 mg of AML were taken and accurately weighed. Average weight was determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 8 mg TEL and 1 mg AML was transferred to volumetric flask of 10 ml capacity, 1 M HCl was added to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with 1M HCl. The above solution was filtered through Whatmann filter paper (No. 41). From the filtrate 0.1 ml was transferred into volumetric flask of 10 ml capacity. Volume was made up to the mark with 1 M HCl to give a solution containing 8 µg/ml TEL and 1 µg/ml AML. These solutions were used for the estimation of drugs.

Method validation. The method was validated for linearity, sensitivity, selectivity, precision and accuracy for each compound as per International Conference on Harmonization (ICH) guidelines.¹⁶

Linearity. The prepared standard dilutions of TEL (4-14 µg/ml) and AML (1-6 µg/ml) quantified by first derivative synchronous spectrofluorimetric technique and fluorescence intensities were recorded. The calibration curve was constructed by plotting the analyte intensity against the respective concentration.

Sensitivity. The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on standard calibration curve.

Precision. Precision of the method was determined by intra-day and inter-day precision variations as per ICH guidelines. For both intra-day and inter-day precisions, the samples containing

TEL (6, 10 and 14 µg/ml) and AML (2, 4 and 6 µg/ml) were analyzed six times on the same day (intra-day precision) and for three consecutive days (inter day precision). The % RSD was calculated.

Accuracy. The accuracy was carried out by recovery studies using standard addition method; known amount of standard drug was added to pre analyzed sample of TEL and AML in according to 80, 100 and 120% levels of labeled claim and then subjected to the proposed method. The experiment was conducted in triplicate. The percentage recovery and percentage relative standard deviation (% RSD) were calculated for each concentration.

RESULTS AND DISCUSSION

Method optimization. TEL molecule contains polycyclic aromatic systems like two benzimidazole rings and benzoic acid, in which more π electrons are available to exhibit fluorescence. Similarly, AML show fluorescence in the presence of electron donating group $-\text{NH}_2$. Different solvent systems were tested in order to find the best conditions like solubility, fluorescence activity, stability and spectral discrimination (clear separation) of both the drugs. TEL exhibits native fluorescence at emission wave length of 370 nm after excitation at 298 nm and similarly AML exhibits fluorescence at emission

wave length of 461 nm after excitation at 374 nm in 1M HCl (Figure 2). It was revealed that, the fluorescence spectra of these drugs overlap considerably and as a result, the conventional spectrofluorimetric method did not permit the simultaneous determination of both the drugs. It was necessary to record the synchronous spectra of TEL and AML corrected for the blank signal and maintaining a constant interval between the emission and excitation wavelengths of 50 nm (Figure 3). There was large overlap of the spectra, the quantification of TEL and AML by synchronous spectrofluorimetry was not possible. This overlap was highly discriminated by using first derivative synchronous spectrofluorimetric method, which was used to choose the suitable wavelengths that make the estimation proportional to TEL and AML concentrations with the “zero crossing”. The first derivative synchronised spectrum of TEL has zero intensity at 458 nm, whereas AML gives significant derivative response, while the derivative spectrum of AML has zero intensity at 675 nm, while TEL gives significant derivative response (Figure 4). Therefore, 458.0 nm was selected for estimation of AML and 675 nm was chosen for the estimation of TEL in synthetic mixture and tablet dosage form.

Table 1. Optimum conditions of for the proposed method of analysis.

Parameter	Amlodipine besylate	Telmisartan
Emission wavelength (nm)	458	675
Beer's Law Limit (µg/ml)	1-6	4-14
LOD (µg/ml)	0.013	0.070
LOQ (µg /ml)	0.044	0.233
Regression equation,*		
Slope (b)	0.022	0.0214
Intercept (a)	0.0007	0.0081
Standard deviation of slope	0.0012	0.0025
Standard deviation of intercept	0.0002	0.0015
Correlation coefficient	0.9991	0.9975

*Y= a+bX. Y is the absorbance, X is the concentration in µg/ml, a is the intercept and b is the slope.

Table 2. Results of analysis of marketed tablets by the proposed method.

Tablet brand name	Telmisartan			Amlodipine besylate		
	Label claim (mg)	Quantity found* (mg) AM \pm SD	% RSD	Label claim (mg)	Quantity found* (mg) AM \pm SD	% RSD
Telsartan-AM	40	40.09 \pm 0.257	0.64	5	4.926 \pm 0.063	1.289
Sartel-AM	40	39.85 \pm 0.057	0.143	5	4.991 \pm 0.0144	0.288

* Mean of three determinations.

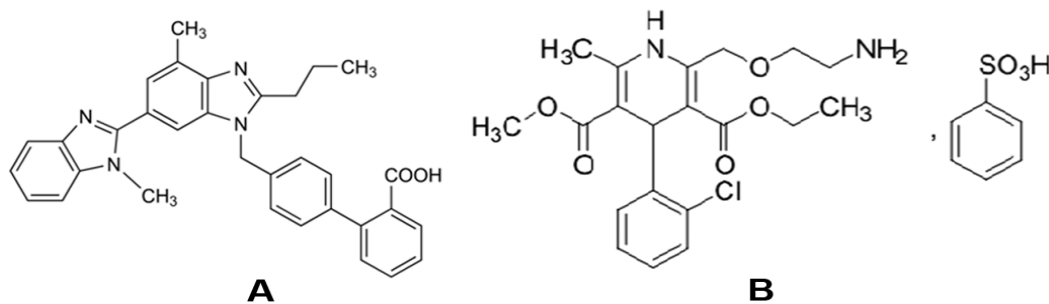


Figure 1. Chemical structure of telmisartan (A) and amlodipine besylate (B).

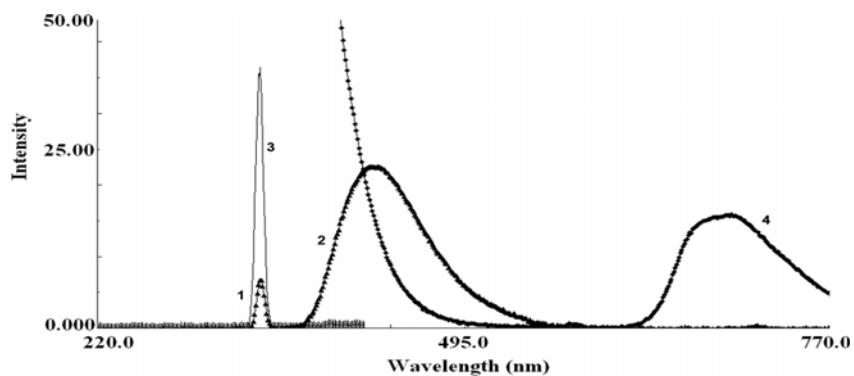
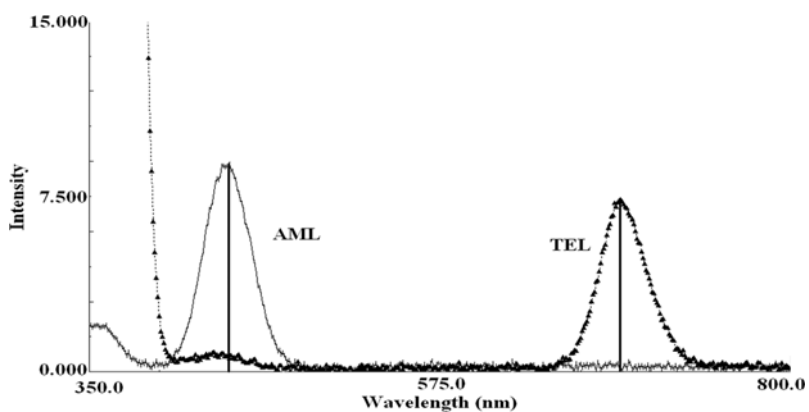
Figure 2. Excitation (1, 3) and emission (2, 4) spectra of amlodipine besylate (4 μ g/ml) and telmisartan (4 μ g/ml).

Figure 3. Synchronous fluorescence spectra of amlodipine besylate (AML) and telmisartan (TEL) in 1M HCl solution.

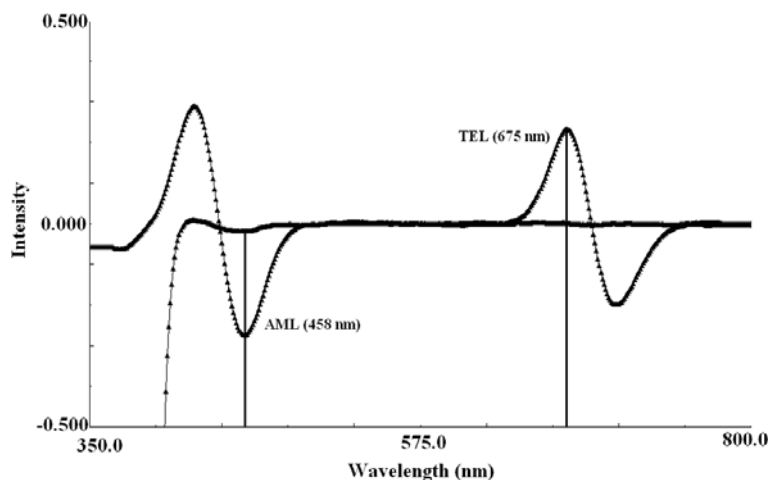


Figure 4. First order synchronous emission spectra of amlodipine besylate (AML) and telmisartan (TEL) in 1 M HCl solution.

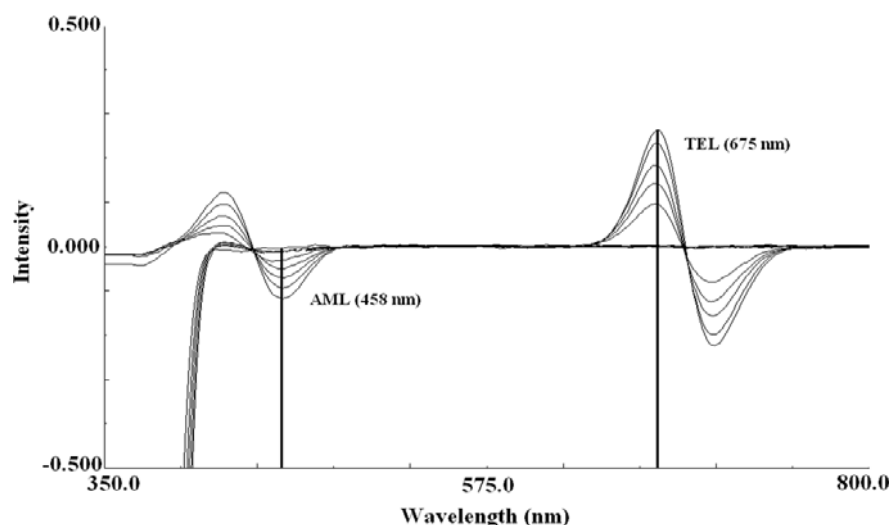


Figure 5. First derivative synchronous linearity spectrum of amlodipine besylate (AML) and telmisartan (TEL).

Method validation. The linearity was evaluated by the least square regression method. The responses for TEL at 675 nm were found to be linear in the concentration range of 4-14 $\mu\text{g/ml}$ with a correlation co-efficient (r) value of 0.997. Similarly the responses for AML at 458 nm were linear in the concentration range of 1-6 $\mu\text{g/ml}$ with a correlation coefficient (r) value of 0.999. The range of linearity spectra are shown in Figure 5. Optimum conditions of the proposed method are mentioned in Table 1. The % recoveries of TEL and AML were found to be in the range 99.5 - 101.7 and 98.60 - 101.24, respectively and the % RSD at each concentration level was less than 1.84, thus indicated the accuracy

of the method. There was no significant difference between the % RSD values of intra-day and inter-day precision, which revealed that the method was reproducible. The sensitivity of the method as indicated by LOD and LOQ values of the proposed method, reported in Table 1, indicated the high sensitivity of the method.

Assay of marketed tablet formulations. The proposed method was applied to the assay of commercially available tablets (Telsartan-AM and Sartel-AM) containing telmisartan (40 mg) and amlodipine besylate (5 mg). The results obtained for TEL and AML were compared with the corresponding labeled amounts and reported in

Table 2. The % potency in the commercial formulations was found to be in the range 99.60 – 100.22 for TEL and 98.40 – 99.80 for AML by the proposed method. The % RSD for the formulations (Telsartan-AM and Sartel-AM) was less than 1.28 and 0.28 respectively.

CONCLUSION

In the present study, a new simple, sensitive and time saving first derivative synchronous spectrofluorimetric method has been developed for simultaneous quantification of TEL and AML in binary mixture and pharmaceutical dosage forms. This spectrofluorimetric method has been found to be superior, because of its highly specific spectral discrimination, readily available solvent, economical, eco-friendly and lack of extraction procedure. The assay values were in good concurrence with their respective labeled claim, which suggested no interference of formulation excipients in the estimation and the obtained results from validation proved the proposed method to be scientifically sound. These advantages suggest that the developed method can be routinely employed in quality control of TEL and AML in tablet dosage form.

ACKNOWLEDGEMENT

The authors are thankful to the management of Gokaraju Rangaraju College of Pharmacy for providing facilities for this research work.

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