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SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR AMLODIPINE BESYLATE AND SIMVASTATIN IN BULK AND TABLET DOSAGE FORM USING ABSORPTION RATIO METHOD

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ABSTRACT

A simple, economic and accurate absorption ratio method was developed for the simultaneous estimation of Amlodipine besylate and Simvastatin in bulk and tablet dosage form. 0.1M HCL in 1% Sodim Lauryl Sulphate was used as a diluent. The absorptions were observed at 249.78nm and 367.85nm which were selected based on overlap spectra of Amlodipine besylate and Simvastatin. The Linearity range was found to be 5-30ug/ml (r^2 =0.999) at 249.78nm and (r^2 = 0.996) at 367.85nm. The proposed method was validated. The reports was expressed that the proposed method was found to be simple, precise, accurate and rapid for the simultaneous estimation of Amlodipine besylate and Simvastatin in bulk and tablet dosage form using absorption ratio method.

KEYWORDS: Absorption ratio, Amlodipine besylate, Simvastatin, Hydrochloric acid, Sodim Lauryl Sulphate .

INTRODUCTION

Simvastatin (SIM) is chemically as (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7- dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate. It is used as an Anticholesteremic Agents. Simvastatin is a lipid-lowering agent that is derived

synthetically from the fermentation of Aspergillus terreus. It is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (hydroxymethylglutaryl COA reductases), which is the rate-limiting enzyme in cholesterol biosynthesis. It may also interfere with steroid hormone production. Due to the induction of hepatic LDL receptors, it increases breakdown of LDL cholesterol[1,2]. The molecular formula is $C_{25}H_{38}O_5$ and the structural formula is in figure 1. SIM is soluble in water. It has a molecular weight of 418.566 g/mol. Literature survey revealed that various analytical methods such as UV-Visible (Vis) spectrophotometry[3,4] High performance liquid chromatography[5,6] and High performance thin layer chromatography methods[7,8] have been reported for estimation of SIM from its formulations and biological fluids. Amlodipine Besylate (AML) is a long-acting 1,4-dihydropyridine calcium channel blocker. 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. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase. Some studies have shown that amlodipine also exerts inhibitory effects on voltage-gated N-type calcium channels. N-type calcium channels located in the central nervous system may be involved in nociceptive signaling and pain sensation. Amlodipine is used to treat hypertension and chronic stable angina[1]. It is chemically known as 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. 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. Literature survey revealed that various analytical methods such as UV-Visible (Vis) spectrophotometry[9], Spectrofluorimetry[10], High performance liquid chromatography and High performance thin layer chromatography[11-15] are available for the estimation of AML alone or with some other drugs in formulations.

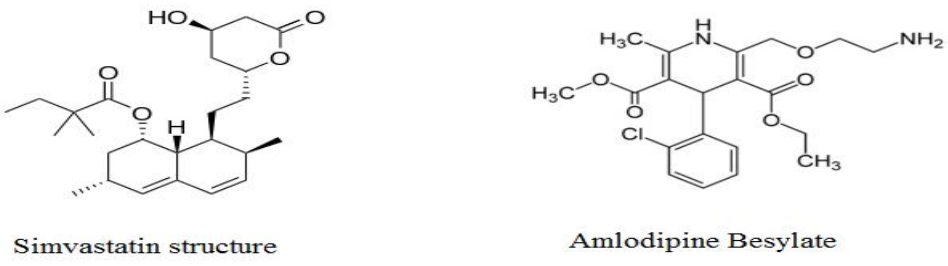

Figure 1: Structure of Amlodipine besylate and Simvastatin

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters which should be followed in the development and validation. A survey of literature reveals that simultaneous analytical methods are not available for the drug combination SIM and AML, even though very few methods of individual estimation of above drugs are available. Hence it is proposed to develop new methods for the assay of SIM and AML in pharmaceutical dosage forms adapting UV Visible spectrophotometry. The objective of the proposed method was to develop simple and accurate methods for the determination of SIM and AML simultaneously using absorption ratio method by UV-Spectrophotometry in pharmaceutical dosage forms.

MATERIALS AND METHODS

AML and SIM obtained from Aurabindho Laboratories. A commercial sample AML and SIM tablets were procured from local market and used within their shelf-life period. Hydrochloric acid from S.D. Fine Chemical Limited, India was of Pharmaceutical or analytical grade, sodium lauryl sulphate from Qualigens Fine Chemicals, India was of pharmaceutical or analytical grade. Quantitative estimation was performed on Labindia UV 3000+ and Elico SL 210 double beam UV visible spectrophotometers with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto. Labindia UV Win software was used along with quartz cuvette for the wavelength prediction. To develop a suitable and robust absorption ratio method for the determination of AML and SIM, different diluents like ethanol, 0.1N NaOH etc., were tried based on the solubility and functional group present in the compound. Finally 0.1M HCl in 1% sodim lauryl sulphate was selected due its reproducible results. Absorbance were measured at selected wavelength (249.78nm and 367.85nm) based on the overlap spectrum of both drugs. The data were collected and analyzed with software in a computer system.

Preparations

Stock solution of AML (200mcg/ml) was prepared by dissolving 10 mg of AML in 50 ml of volumetric flask containing 20ml of 0.1M HCl in 1%SLS. The solution was sonicated for about 15 minutes and then made up to volume with mobile phase. From the stock solution, 1ml was pipetted out and transferred into the 10ml volumetric flask to get 20µg/ml concentration. Same procedure followed for SIM standard. The final solutions of both

standard drugs solutions were undergone for scanning and overlapped each other. Two wavelengths were selected. Among the two, 367.85nm is a λmax of AML and 249.78 nm is an isobestic point. Then the absorbance was measured at 249.78nm and 367.85nm and calculated the absorptivity.

Preparation of standard mixture

From 200µg/ml of AML and SIM standard stock solutions, 1ml was pipette out individually and mixed in 10ml volumetric flask then it was made up to the mark with 0.1M HCl in 1%SLS. Absorbance was measured at selected wavelengths (249.78nm and 367.85nm).

Preparation of tablet mixture

20 tablets were weighed and powdered. The amount of powder equivalent to 15mg of AML and 25mg of SIM were weighed and transferred into the 100ml of volumetric flask containing 20 ml of 0.1m HCl in 1%SLS. The solution was sonicated for about 20 minutes and then made up to volume with mobile phase. The solution was filtered. From the filtrate, 2ml was pipetted out and transferred into the 10ml volumetric flask then made up to the mark with 0.1m HCl in 1%SLS. A typical overlap spectrogram of standard SIM and AML was shown in figure 2. The amount of drug present in pharmaceutical formulation was calculated through the following formula: Cy=(A1/ax1)-Cx Cx=((Qm-Qy)/Qx-Qy))(A1/ax1), where, Cy is a concentration of in Simvastatin mixture; Cx is a concentration of Amlodipine besylate in mixture; Qx(absorption ratio of drug 1)=ax2/ax1; Qy(absorption ratio of drug 2)=ay2-ay1; Qm(absorption ratio of mixture=A2/A1 where A1 is absorption at 249.78nm in mixture; A2 is absorption at 367.85nm in mixture and a is an absorptivity.

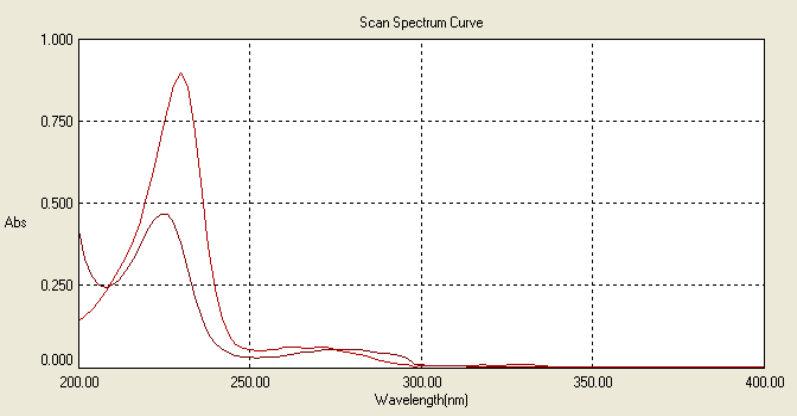

Figure 2: Overlap spectrogram of Amlodipine besylate and Simvastatin.

Validation

The described method has been validated for the assay of SIM and AML using following parameters. Linearity was studied to find out the relationship of concentration with absorbance. The different concentrations of SIM and AML mixtures (10 to 20µg/ml of each drug in the mixture) were taken for linearity. The all solutions were undergone for scanning and measured the absorbance at 249.78nm and 367.85nm. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/mL). Alternatively, the corresponding regression equation was derived. Precision was studied to find out variations in the test methods of mixtures SIM and AML (20µg/ml) on the same day and on different day by using different Instrument (Elico SL210, Labindia UV 3000+) (Ruggedness). The precision of each method was ascertained separately from the absorbance obtained by actual determination of six replicates of a fixed amount of drug (20µg/ml). Precision and Ruggedness were done on the same day and the different day respectively and the %RSD was calculated for each. The accuracy of the method was shown by analyzing the model mixtures consists 80%, 100% and 120% of SIM and AML. After the measurement, the Amount found, Amount added for SIM and AML and individual recovery were calculated. LOD and LOQ were calculated based on the calibration curve method.

RESULTS AND DISCUSSION

An absorption ratio method procedure was proposed as a suitable method for the analysis of drugs AML and SIM in dosage forms. The wavelength was found to be 249.78nm and 367.85nm. The regression equation for the method at 249.78nm was found to be y=0.048x+0.0241 (r^2=0.999), where 0.048is a slope; 0.0241 is an Intercept; r^2 is correlation coefficient (0.999) and found to be linear over Beer's Range 5- 30µg/ml respectively. The regression equation for the method at 367.85nm was found to be y=0.0108x+0.0049 (r^2=0.996), where 0.0108 is a slope; 0.0049 is an Intercept; r^2 is correlation coefficient (0.996) and found to be linear over beer's range 5-30µg/ml respectively. The Linearity graph of SIM and AML mixtures was shown in figure 3.

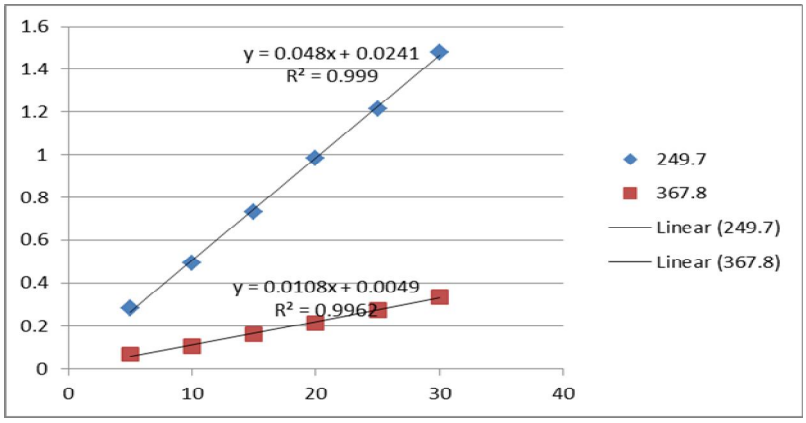

Figure 3: Linearity graph for absorption Ratio method for estimation of Amlodipine besylate and Simvastatin.

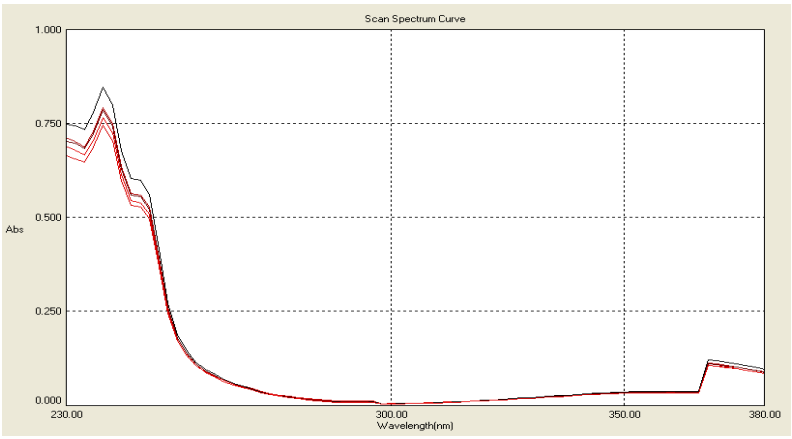

Figure 4: Overlap spectra of different concentration of mixture of Amlodipine besylate and Simvastatin for Linearity

Figure 4 expressed the overlap spectra of different concentration of mixture of SIM and AML for Linearity. The percentage of purity of SIM and AML in tablet dosage form was 88.96% and 94.80% respectively. 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. Precision data were present in table 1.

Table 1: Data for Precision

	Absorbance at 249.78nm	Absorbance at 367.85nm	Absorption ratio	Concentration of SIM	Concentration of AML
SIM standard	0.252	0.114	0.452381		
AML standard	0.252	-0.00025	-0.00099		
mixture(m1)	0.489	0.108	0.220859	0.00095	0. 000991
mixture(m2)	0.485	0.106	0.218557	0.000932	0.000993

mixture(m3)	0.49	0.104	0.212245	0.000915	0.00103
mixture(m4)	0.491	0.105	0.213849	0.000923	0.001025
mixture(m5)	0.491	0.108	0.219959	0.00095	0.000999
Mean	0.489167	0.106667		0.000938	0.001003
SD	0.002229	0.001966		1.727505	1.968057
%RSD	0.455592	1.843485		1.835075	1.951528
SIM= Simvastatin; AML= Amlodipine; SD= Standard deviation; %RSD= Percentage Relative Standard deviation.					

In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 80%, 100% and 120% of sample solution of SIM and AML and along with 10µg/ml of bulk standard solution within the linearity ranges. The percentage recoveries were found to be 96.980, 90.688 and 9.02%w/w for 80%, 100% and 120% of SIM and 97.647, 91.626 and 90.532%w/w for 80%, 100% and 120% of AML. Accuracy data were present in Table 2. The percent recoveries values indicate less interference from excipients used in formulation. LOD for SIM and AML was found to be 0.7721µg and 0.7848µg respectively. LOQ for SIM and AML was found to be 2.3398 µg and 2.3768 µg respectively.

Table 2: Data for Accuracy

	Absorbance at 249.78nm	Absorbance at 367.85nm	Absorption ratio	Concentration of SIM	Concentration of AML	% recovery for SIM	% recovery for AML
Accuracy 1(80%)	0.686	1.085	1.5816	0.3297	0.3320	96.980	97.647
Accuracy 2(100%)	0.756	1.195	1.5807	0.3627	0.3627	90.688	91.626
Accuracy 3(120%)	0.806	1.361	1.5816	0.4164	0.4164	90.020	90.532

CONCLUSION

The presented method was precise, sensitive and accurate. The advantages of proposed method were its simple procedure for sample preparation. The good recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of SIM and AML in pharmaceuticals.

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