Simultaneous Spectrophotometric Determination of Atorvastatin Calcium and Amlodipine Besylate in tablets

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Two simple, accurate and precise methods for simultaneous estimation of atorvastatin calcium and amlodipine besylate in combined dosage form have been described. First method employs formation and solving of simultaneous equations using 245 nm and 363 nm as two analytical wavelengths. Second is dual wavelength method, which uses the difference of absorbance value at 259.9 nm and 354 nm for estimation of atorvastatin calcium and absorbance at 363 nm for amlodipine besylate. Fifty percent methanol was used as solvent, in which atorvastatin calcium and amlodipine besylate shows linearity in the range of 0-40 µg/ml and 0-20 µg/ml, respectively. Standard deviation was <1.5 in the assay of tablets. Methods were validated as per ICH norms and accuracy, precision, repeatability and robustness was found to be with in the acceptable limit.

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The combination of atorvastatin calcium (AC)[1] and amlodipine besylate (AB)[2] is widely used in the treatment of hypertension. Reports are available for estimation of AB in the combined formulation using HPTLC, Spectrometry and of AC by HPLC, HPLC-MS[3]. Present paper describes two spectrophotometric methods for simultaneous estimation of AC and AB using 50% methanol as solvent, after considering the solubility, stability and spectral characteristics of both the drugs.

UV/Vis double beam spectrophotometer, Shimadzu-1700 UV with 1 cm matched quartz cells and electronic balance, MEL 200 were used. Reference standard of atorvastatin calcium and amlodipine besylate was gifted from Ranbaxy Pvt. Ltd., Indore. Methanol AR grade (Merck Pvt. Ltd.) and distilled water was used for analysis.

Standard stock solution A (1000 µg/ml) was prepared by taking accurately weighed 100 mg of AC and AB separately into 100 ml volumetric flask and dissolved in 25 ml of 50% methanol, sonicated for 10 min and finally volume was made up to the mark with the solvent. For selection of analytical wavelengths for Simultaneous equation method[4] (Method 1), standard solution of 40 µg/ml of AC and AB were scanned in the wavelength range of 200-400 nm. Wavelengths 245 nm and 363 nm which are the λ max of AC and AB, respectively were selected for formation of the simultaneous equations.

The absorptivities (A1%, 1 cm) of both the drugs at the wavelengths were determined. The absorbance and absorptivity values at the particular wavelengths were substituted in the following equations to obtain the concentrations: $C_x = A^2a^1_y - A^1a^2_y / a^2_x a^1_y - a^1_x a^2_y$ (1), $C_y = a^2_x A^1 - a^1_x a^2 / a^2_x a^1_y - a^1_x a^2_y$ (2), where A^1, A^2 are absorbance of the mixture, a^1_x and a^2_x are absorptivities of X, a^1_y, a^2_y denote absorptivities of Y at 245 nm and 363 nm respectively, C_x is the concentration of AC and C_y is the concentration of the AB.

For dual wavelength method (Method 2) from the overlain spectra of AC and AB, 363 nm was taken as the wavelength for estimation of AB, as AC shows no absorbance at this wavelength. The wavelengths selected for estimation of AC are 259.9 and 354 nm, where AB shows same absorbance.

Series of mixed standards were prepared from the separate sub stock B (100 µg/ml) of different concentrations in the range 0-40 µg/ml for AC and 0-20 µg/ml for AB. Mixed standards were scanned and calibration curve was plotted in between absorbance difference at the selected wavelength and concentration for estimation of AC and absorbance at 363 nm against concentration for AB.

Tablet estimation was done on of two brands, Starcad (Lupin labs, label claim 10 mg of AC and 5 mg of AB) and Lipikind-AN (Mankind labs, label claim 10 mg of AC and 5 mg of AB). Twenty tablets were weighed and finely powered amount equivalent to 10 mg of AC was taken in 10 ml volumetric flask. This was then dissolved in 5 ml methanol by sonication for about 5 min. The volume was made up to the mark by water and was filtered through Watmann filter paper (No. 41). The filtrate was further diluted to get final concentrations of both drugs in the linearity range. Absorbance was noted at the selected wavelengths and concentrations were determined. The analysis was carried out in set of 6 replicate and mean % label claim was determined and the results were statistically validated (Table 1).

Linearity of method was validated by calculating response ratio and plotted against concentration which should be a straight line. Accuracy of the method was determined by recovery studies by adding definite amount of standard drug to tablet sample and percent recovery was calculated. Precision determination was done as intermediate precision and repeatability by analyzing six replicates each intra day and inter day and relative standard deviation was calculated. Also robustness of the method was performed by changing the percentage of solvent from 50 to 75% and relative standard deviation was noted.

The overlain spectra of both drugs showed that the peak are well resolved thus satisfying the criteria for obtaining maximum precision based on absorbance ratios for simultaneous equation method which lies outside the range of 0.1-2.0. The spectra of AB show two distinct peaks, one at 239 nm and other at 363 nm. At second wavelength AB shows better linearity. Hence, for simultaneous equation method, this wavelength was selected for the determination of AB. Since only one prominent peak exists for AC at 245 nm, the same was used for its determination. Absorbance was determined at both wavelengths, calibration curves were plotted and regression analysis was carried out. The absorptivity was then calculated and along with absorbance values were

TABLE 1: STATISTICAL EVALUATION AND VALIDATION PARAMETERS

Parameters	Simultaneous equation		Dual wavelength	
	Atorvastatin calcium	Amlodipine besylate	Atorvastatin calcium	Amlodipine besylate
Standard deviation*	0.677	1.063	1.034	1.33
% coefficient of validation*	0.663	1.051	1.037	1.33
Standard error*	0.276	0.434	0.422	0.544
Linearity range (µg/ml)	0-40	0-20	0-40	0-20
Correlation coefficient (r^2)	0.9991	0.9995	0.9991	0.9997
Accuracy (%)	102.2	101.16	99.66	100.10
Precision intermediate precision (RSD)	0.294	0.678	0.659	0.867
Repeatability (RSD)	0.343	0.523	0.611	0.734
Robustness (RSD)	0.442	0.358	0.404	0.393

*Mean of 6 determinations

TABLE 2: ASSAY RESULTS OF ATORVASTATIN CALCIUM AND AMLODIPINE BESYLATE IN COMMERCIAL FORMULATIONS

Method	Label claim		Mean amount found* (mg/tab)		Mean % label claim*	
	Atorvastatin calcium	Amlodipine besylate	Atorvastatin calcium	Amlodipine besylate	Atorvastatin calcium	Amlodipine besylate
Simultaneous equation	10	5	10.18	5.06	101.80	101.16
Dual wavelength	10	5	9.96	5.01	99.67	100.10

*Mean of 6 determinations

substituted in the equation 1 and 2 to obtain concentration of drugs in the sample.

For dual wavelength method, the spectra of AC and AB when overlaid indicated that at 259.9 nm and 354 nm, the AB have similar absorbance and therefore absorbance difference at these wavelengths are because of AC only. It was also observed that AB shows a prominent peak at 363 nm while AC has no interference at this wavelength. Hence absorbance difference at 259.9 nm and 354.0 nm and absorbance at 363.0 nm was considered for estimation of AC and AB, respectively.

Both the methods were successfully used to estimate the amounts of atorvastatin calcium and amlodipine besylate present in two of the marketed tablet formulations. The assay of the tablet formulations were done and the results were statistically validated and are found to be in acceptable range having RSD<1.5. The validations of both methods were done as per ICH norms and results obtained are well in the acceptable range RSD<1 (Table 2).

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