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## Development and validation of UV-spectrophotometric methods for simultaneous estimation of amlodipine besylate and clopidogrel bisulfate in bulk and tablet dosage form

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### ABSTRACT

Two simple spectrophotometric methods have been developed for simultaneous estimation of Amlodipine besylate (AB) and Clopidogrel bisulphate (CPS) from tablet dosage form. Method (I) is simultaneous equation method, wavelengths selected are 360 and 270 nm for AB and CPS respectively. Method (II) is the absorbance ratio method, based on the determination of graphical absorbance ratio at two selected wavelengths, one being the isoabsorptive point 329 nm and other being the absorption maxima of amlodipine besylate at 360 nm. Both drugs obeyed Beer's law in the concentration range 5-45 µg/ml, correlation coefficient ( $r^2 < 1$ ). The accuracy and precision were determined and found to comply with ICH guidelines. Both the methods showed good reproducibility and recovery with % RSD in the desired range. The proposed methods can be successfully applied for the routine analysis of both the drugs from tablet dosage form.

**Keywords:** Amlodipine besylate, Clopidogrel bisulphate, UV spectroscopy, Simultaneous Equation method, Absorbance Ratio method.

### INTRODUCTION

Amlodipine besylate (AB) (fig. 1a) is the besylate salt of amlodipine, chemically known as, 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl, 5-methyl ester ( $\pm$ ) monobenzenesulfonate. It is a long-acting, calcium channel blocker. It is used in the treatment of hypertension and angina<sup>[1-6]</sup>. Clopidogrel (CPS) (fig. 1b) is an analogue of ticlopidine, chemically known as, methyl-2-chlorophenyl-(4, 5, 6, 7-tetrahydrothieno [3,2-c] pyridine-5yl) acetate bisulphate. It is an antiplatelet agent, it is used for the long term prevention of atherothrombotic events (myocardial infarction, stroke, peripheral arterial disease, acute coronary syndrome, cardio-vascular death) it is used in the treatment of cardiovascular diseases<sup>[6-8]</sup>. AB is official in USP, BP, EP & IP whereas CPS is official in USP<sup>[1-4]</sup>. The chemical structures of AB & CPS are shown in Fig. 1. Combination drug products of AB and CPS are widely marketed and used in the treatment of hypertension and cardiac disorders. Several analytical methods like UV spectrophotometry, HPLC, HPTLC, UPLC have been reported for estimation of AB & CPS by single drug and also by combining with other drugs. However no method

has been reported till date for the simultaneous estimation of AB & CPS using the UV spectrophotometric method. The present paper describes the development and validation of two analytical methods for simultaneous estimation of AB & CPS by UV spectrophotometry in tablet dosage form, the methods include simultaneous equation method & absorbance ratio method. The proposed methods are optimized and validated as per the ICH guidelines<sup>[10,11]</sup>.

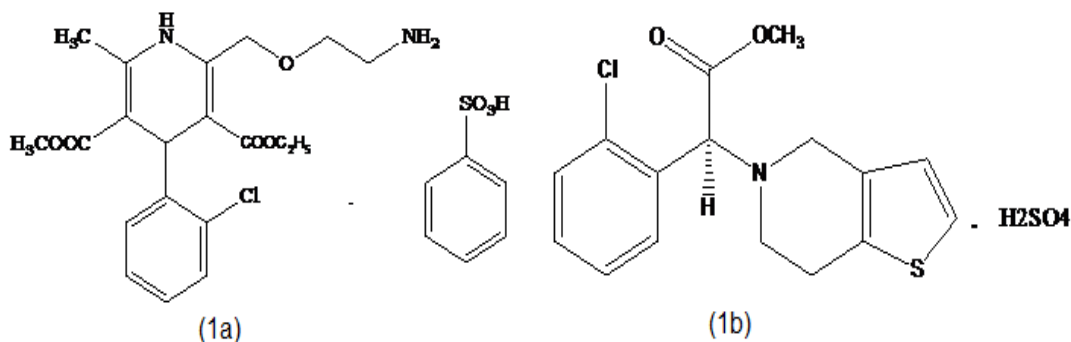


Fig. 1 Chemical structures of the analytes (1a) AB & (1b) CPS

## MATERIALS AND METHODS

### Instrumentation

SHIMADZU double beam UV visible spectrophotometer (model 1800) with 1 cm matched quartz cells were used for all absorbance measurements. Shimadzu AUX 220 balance was used for weighing the samples.

### Reagents

All the chemicals used were of AR grade and obtained from Merck, Mumbai, India Limited. Double distilled water and Whatmann filter paper (0.45 $\mu$ m) were used for filtration. Active pharmaceutical ingredient (API) working standards of amlodipine besylate (AB), clopidogrel bisulphate (CPS) were obtained as gift sample from Lupin Laboratories limited Pune, India and test samples (tablets with composition CPS-75 mg and AB, equivalent to amlodipine-5 mg) were procured from the local market, Pune, India.

### Preparation of phosphate buffer solution

Dissolve 3.4g of potassium dihydrogen phosphate in 900mL double distilled water. Adjust the pH 3.0 with o-phosphoric acid and make up the volume up to 1000 ml with same solution<sup>[9]</sup>.

### Preparation of standard stock solution

Standard stock solutions (1000  $\mu$ g/ml) of AB and CPS were prepared separately by dissolving 100 mg of AB and CPS, respectively in 100 ml methanol.

### Preparation of sample solution

Ten tablets, labeled as containing 5 mg of AB, and 75 mg of CPS together with excipients, were accurately weighed, transferred to a clean and dry mortar and ground into a fine powder. A weight of the powder equivalent to one tablet content (390 mg) was accurately weighed, then transferred to a clean 50 ml volumetric flask, 20 ml of methanol was added, and the flask was attached to a rotary shaker for 10 min to disperse the material completely. The mixture was then sonicated for 10 min and diluted to volume methanol to give a solution containing 100  $\mu$ g/ml concentration of AB and 1500  $\mu$ g/ml concentration of CPS. This solution was filtered through a 0.45 $\mu$ m pore size, Nylon 66 membrane filter and the sample solutions of required concentration are prepared and diluted with potassium dihydrogen phosphate buffer pH 3.

### Calibration Curve Procedure

Aliquots of standard stock solutions of AB and CPS were taken in volumetric flasks and diluted with phosphate buffer to get final concentrations in range of 5-45  $\mu$ g/ml for both the drugs. The solution was scanned in the range of 200 to 400 nm against methanol as blank, the excitation wavelengths were found to be 360nm for AB and 270nm for CPS and iso-absorptive point at 329nm. The calibration curve was obtained by plotting absorbance values at 360nm & 270nm (for method I shown in fig. 2 & 3), & at 329nm & 270nm (for method II shown in fig. 4 & 5) against

amount of standard drug in µg/ml. The absorptivity values of both the drugs were determined at these selected wavelengths.

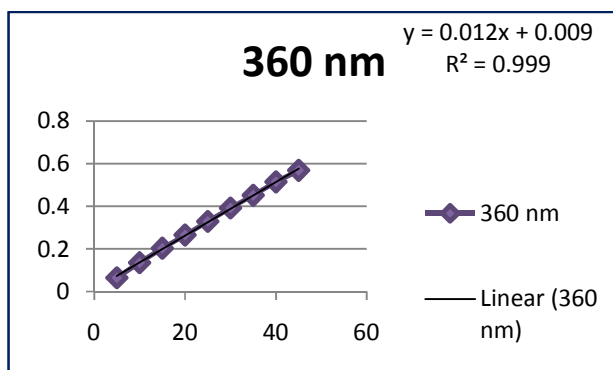


Fig. 2 Standard Calibration curve of Amlodipine Besylate at 360 nm

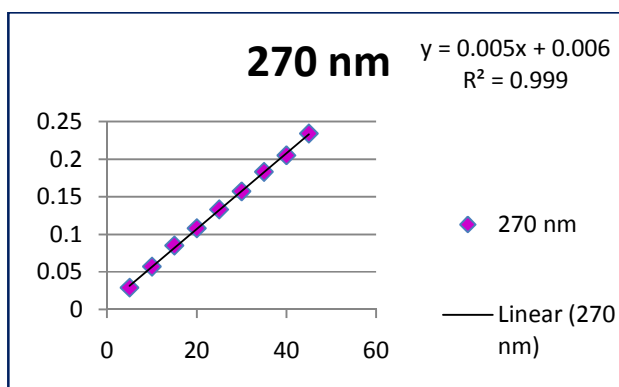


Fig. 3 Standard Calibration curve of Clopidogrel bisulfate at 270 nm

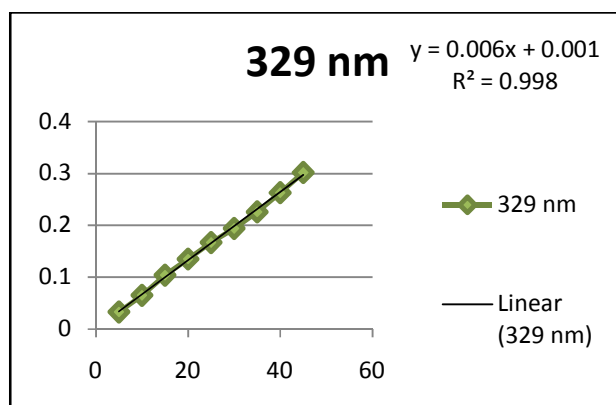


Fig. 4 Standard Calibration curve of Amlodipine Besylate at 329 nm

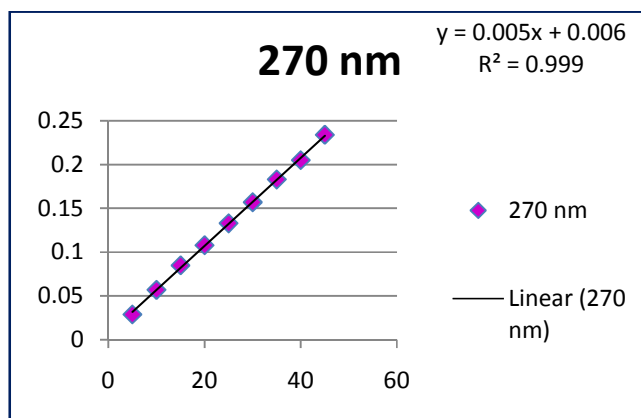


Fig. 5 Standard Calibration curve of Clopidogrel bisulfate at 270 nm

### Method I

Simultaneous equation method uses the absorbances at two selected wavelengths, both being the  $\lambda_{\max}$  of the two drugs. Working standard solutions were scanned in the range of 200-400 nm to determine the  $\lambda_{\max}$  of both the drugs. The  $\lambda_{\max}$  of AB and CPS were found to be 360 nm and 270 nm respectively (fig. 2). Nine standard solutions having concentrations 5, 10, 15, 20, 25, 30, 35, 40, 45  $\mu\text{g/ml}$  for AB & CPS were prepared in potassium dihydrogen phosphate buffer pH 3. The absorbances of resulting solutions were measured at 360 nm and 270 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficient of these two drugs was determined using the calibration curve equation. Two simultaneous equations were formed using these specific absorbance values.  $A_1 = 13.482C_x + 0.586C_y$ ,  $A_2 = 10.156C_x + 5.376C_y$ , where,  $C_x$  and  $C_y$  are concentrations of AB and CPS, respectively, in g/100 ml in sample solution.  $A_1$  and  $A_2$  are absorbances of the sample solution at 360 nm and 270 nm, respectively.

The concentration of  $C_x$  and  $C_y$  can be obtained as,  $C_x = (A_2 a_{y1} - A_1 a_{y2}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$  and  $C_y = (A_1 a_{x2} - A_2 a_{x1}) / (a_{x2} a_{y1} - a_{x1} a_{y2})$ , where  $A_1$  and  $A_2$  are the absorbances of mixture at 360 nm and 270 nm respectively,  $a_{x1}$  and  $a_{x2}$  are absorptivities of AB at 360 nm and 270 nm respectively,  $a_{y1}$  and  $a_{y2}$  are absorptivities of CPS at 360 nm and 270 nm respectively,  $C_x$  and  $C_y$  are concentrations of AB and CPS respectively.

### Method II

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths one at iso-absorptive point and other being the  $\lambda_{\max}$  of one of the two components. From the overlain spectra of two drugs, it is evident that AB and CPS show an iso-absorptive point at 329 nm and the second wavelength used was 270 nm, which is  $\lambda_{\max}$  of CPS (fig. 3). Nine standard solutions having concentration 5, 10, 15, 20, 25, 30, 35, 40, 45  $\mu\text{g/ml}$  for CPS were prepared in phosphate buffer pH 3. The absorbances at 329 nm (iso-absorptive point) and 270 nm ( $\lambda_{\max}$  of CPS) were measured and absorptivity coefficients were calculated using calibration curve.

The concentrations  $C_x$  and  $C_y$  of AB and CPS, respectively in the sample mixture can be calculated using equations  $C_x = [(Q_m - Q_y) / (Q_x - Q_y)] \times A_1 / a_{x1}$  and  $C_y = [(Q_m - Q_x) / (Q_y - Q_x)] \times A_1 / a_{y1}$ . The Q-values and absorptivities for both drugs were calculated as follows,  $Q_m = [\text{Absorbance of sample solution at 270 nm} / \text{absorbance of sample solution at 329 nm} (A_1)]$ ,  $Q_x = [\text{Absorptivity of AB at 270 nm} / \text{absorptivity of AB at 329 nm}]$ ,  $Q_y = [\text{Absorptivity of CPS at 270 nm} / \text{absorptivity of CPS at 329 nm}]$ ,  $a_{x1} = [\text{Absorbance of AB at 329 nm} / \text{concentration of AB in g/100 ml}]$ ,  $a_{y1} = [\text{Absorbance of CPS at 270 nm} / \text{concentration of CPS in g/100 ml}]$ , where,  $Q_x$  and  $Q_y$  are Q values of AB and CPS, respectively,  $a_{x1}$  and  $a_{x2}$  are absorptivities at isoabsorptive point for AB and CPS, respectively. These values were found to be  $Q_x = 1.4013$ ,  $a_{x1} = 7.25$ ,  $Q_y = 5.2952$ ,  $a_{y1} = 1.016$ .

For method I the absorbances of sample solutions were measured at 360 nm and 270 nm and the concentration of two drugs in the sample were determined using Eqns. (1) and (2) (method I). For method II, the absorbances of the sample solution  $A_1$  and  $A_2$  were measured at 329 nm (iso-absorptive point) and 270 nm ( $\lambda_{\max}$  of CPS) respectively and ratio of absorbance were calculated which was known as  $Q_m$ . Relative concentrations of two drugs were calculated using equations (3) and (4). The results of analysis of tablet formulation are shown in Table 1.

**Table 1. Result of Analysis of AB & CPS in tablet formulation**

Method	mg/tablet				% of label claim	
	AB	CPS	AB	CPS	AB	CPS
	Label	Label	Obtained	Obtained		
Method I (SE)	5	75	5.001	75.309	100.02	100.412
Method II (AR)	5	75	5.032	75.046	100.64	100.06

\*Average of six estimations, SE- simultaneous equation, AR-absorbance ratio

### Precision

Precision of the methods was determined by performing interday variation, intraday variation and repeatability studies. In interday variation, the absorbance of sample solutions of AB and CPS (10 µg/ml) were measured on three consecutive days. In intraday variation, the absorbances were measured three times in a day. In repeatability study, six determinations of concentration (10µg/ml) of both the drugs were analyzed. The results are shown in Table 2.

**Table 2. Validation Parameters**

Parameters	Simultaneous Equation Method		Absorbance Ratio Method	
	AB	CPS	AB	CPS
Linearity Range (µg/ml)	5 – 45	5 – 45	5 – 45	5 – 45
Correlation Coefficient	0.999	0.999	0.998	0.999
Precision (%RSD)				
Repeatability(n = 6)	0.30 – 0.91	0.07 – 0.85	0.07 – 0.89	0.07 – 0.85
Intraday(n = 6)	0.32 – 0.90	0.14 – 0.74	0.43 – 0.78	0.07 – 0.087
Interday(n = 6)	0.52 – 0.88	0.34 – 0.52	0.47 – 0.54	0.34 – 0.52
% Recovery (%)	99.09 – 101.5	98.74 – 100.7	98.79 – 101.4	99.48 – 101.6

### Accuracy

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels. A known amount of drug was added to pre-analyzed tablet powder and percentage recoveries were calculated. The results are shown in Table 3.

**Table 3. Recovery Studies**

Name of Drug	Amount of Std. added (µg/ml)	Method I		Method II	
		% Recovery*	SD	% Recovery*	SD
AB	1.6	99.09	0.004	99.96	0.006
	2	99.56	0.005	98.79	0.004
	2.4	101.54	0.003	101.4	0.003
	24	98.74	0.005	99.48	0.004
CPS	30	100.18	0.004	100.26	0.006
	36	100.72	0.003	101.36	0.003

\*Average of three estimations

### LOD & LOQ Determination

The sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantitation (LOQ). The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level. The results are shown in Table 4. The proposed methods were validated as per ICH guideline.

**Table 4. LOD & LOQ Results**

Name of Drug	Simultaneous Equation Method		Absorbance Ratio Method	
	LOD	LOQ	LOD	LOQ
AB	0.159	0.481	0.162	0.366
CPS	0.381	1.154	0.491	1.111

## RESULTS AND DISCUSSION

For Method I under the established conditions, Amlodipine besylate and Clopidogrel bisulfate showed good correlation with Beer's law over the concentration range from 5-45µg/ml at excitation wavelengths 360nm and 270nm with the regression equation  $y = 0.012x + 0.009$  &  $y = 0.005x + 0.006$  for AB & CPS respectively. The correlation coefficient ( $r^2$ ) was found to be 0.999 for both the drugs. A relative standard deviation of 1.204 % and 1.35% was observed on analysis of six replicate samples at excitation wavelengths 360nm and 270nm respectively.

The percent recovery studies revealed that the value lies between 99.09 % - 101.54 % and 98.74 % - 100.72 % at wavelengths 360nm and 270nm respectively. Results of recovery studies demonstrated that the proposed method was highly accurate. Both inter-day as well as intra-day precisions were carried out in different concentration of the solutions and the relative standard deviation (RSD) was found to be less than 2.0. Limit of detection was found to be 0.159 and 0.381µg/ml and limit of quantification was found to be 0.481 and 1.154µg/ml for AB & CPS respectively. Results obtained confirmed the ruggedness of the method.

For Method II under the established conditions, Amlodipine besylate and Clopidogrel bisulfate showed good correlation with Beer's law over the concentration range from 5-45µg/ml at excitation wavelengths 329nm (iso-absorptive point) and 270nm with the regression equation  $y = 0.006x + 0.001$  &  $y = 0.005x + 0.006$  for AB & CPS respectively. The correlation coefficient ( $r^2$ ) was found to be 0.998 & 0.999 respectively. A relative standard deviation of 0.706 % and 0.39% was observed on analysis of six replicate samples at excitation wavelengths 329nm and 270nm respectively. The percent recovery studies revealed that the value lies between 98.79 % - 101.4 % and 99.48 % - 101.36 % at wavelengths 329nm and 270nm respectively. Results of recovery studies demonstrated that the proposed method was highly accurate. Both inter-day as well as intra-day precisions were carried out in different concentration of the solutions and the relative standard deviation (RSD) was found to be less than 2.0. Limit of detection was found to be 0.162 and 0.491µg/ml and limit of quantification was found to be 0.366 and 1.111µg/ml for AB & CPS respectively. Results obtained confirmed the ruggedness of the method.

### CONCLUSION

Proposed UV methods are specific, accurate and precise for the simultaneous determination of Amlodipine besylate and Clopidogrel bisulphate from pharmaceutical dosage form. The described methods are suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

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