Quantitative Analysis of Clopidogrel Bisulphate and Aspirin by First Derivative Spectrophotometric Method in Tablets

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A simple, accurate and precise spectrophotometric method has been developed for simultaneous estimation of clopidogrel bisulphate and aspirin by employing first order derivative zero crossing method. The first order derivative absorption at 232.5 nm (zero cross point of aspirin) was used for clopidogrel bisulphate and 211.3 nm (zero cross point of clopidogrel bisulphate) for aspirin.Both the drugs obeyed linearity in the concentration range of 5.0 µg/ml to 25.0 µg/ml (correlation coefficient r^2<1). No interference was found between both determined constituents and those of matrix. The method was validated statistically and recovery studies were carried out to confirm the accuracy of the method.

Key words: Aspirin, clopidogrel bisulphate, drug analysis, derivative spectrophotometry

Clopidogrel bisulphate (CPS) (methyl-2-chlorophenyl-(4,5,6,7-tertrahydrothienol[3,2-c]pyridine-5yl) acetate bisulphate) and aspirin (ASP) are used in the treatment of cardiovascular diseases. CPS is used as a platelet inhibitor and aspirin as a cyclooxygenase inhibitor[1]. CPS is not official in any of the pharmacopoeias[2-4]. Literature survey revealed several Spectrophotometric[5] and HPLC[6-9] methods for estimation of aspirin, whereas only a few HPLC[10-12] methods are available for clopidogrel bisulphate. A spectrophotometric method[13] was reported recently for simultaneous analysis of ASP and CPS after hydrolyzing the drugs.

Reference standards of CPS and ASP were obtained as gift samples from Lupin Laboratories SIDCO industrial Complex Jammu. AR grade methanol from Qualigens, Mumbai was used as solvent for preparing solutions. The solution of 0.1N HCl was prepared in double distilled water as per IP 1996 procedure. A Shimadzu UV/Vis 1601 double beam spectrophotometer with a fixed slit width (2 nm) and 1 cm matched quartz cells was used for all the spectral measurements. Standard stock solutions (100 µg/ml) of CPS and ASP were prepared by separately

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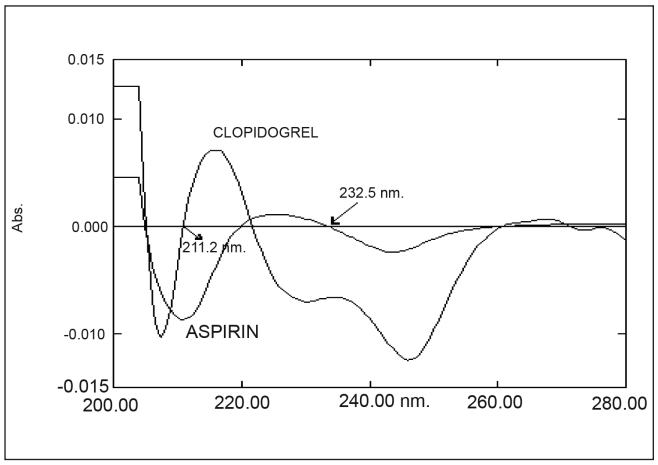

Fig. 1: First order derivative overlain spectra
Clopidogrel (CPS, 10 µg/ml) and aspirin (ASP, 10 µg/ml)

dissolving 10mg each of CPS and ASP, respectively in 100 ml methanol. Suitable aliquot of standard stock solutions were diluted with 0.1N HCl. to obtain solutions of CPS (10 µg/ml) and ASP (10 µg/ml) and scanned in spectrum mode against solvent blank over the range of 200 to 400 nm. The absorption spectra thus obtained were derivatised from first to fourth order. First order derivative spectrum was selected for analysis of both the drugs. From the overlain spectra of both the drugs (fig.1) wavelengths selected for quantitation were 232.5 nm (zero cross point of ASP) for CPS and 211.3 nm (zero cross point of CPS) for ASP.

The standard stock solutions of CPS and ASP were

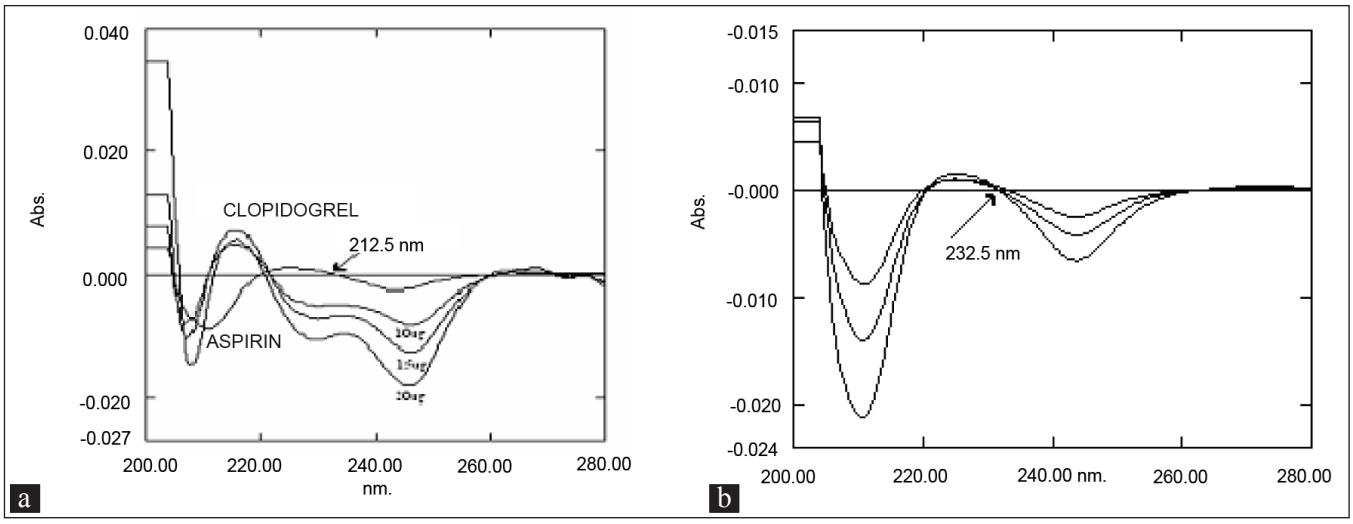

Fig 2: First derivative spectra
(a) First derivative spectra for ASP (10 µg/ml) and CPS (10, 15, 20 µg/ml); (b) First derivative spectra for ASP (10, 15 and 20 µg/ml) and at 232.5 nm D_1 =0

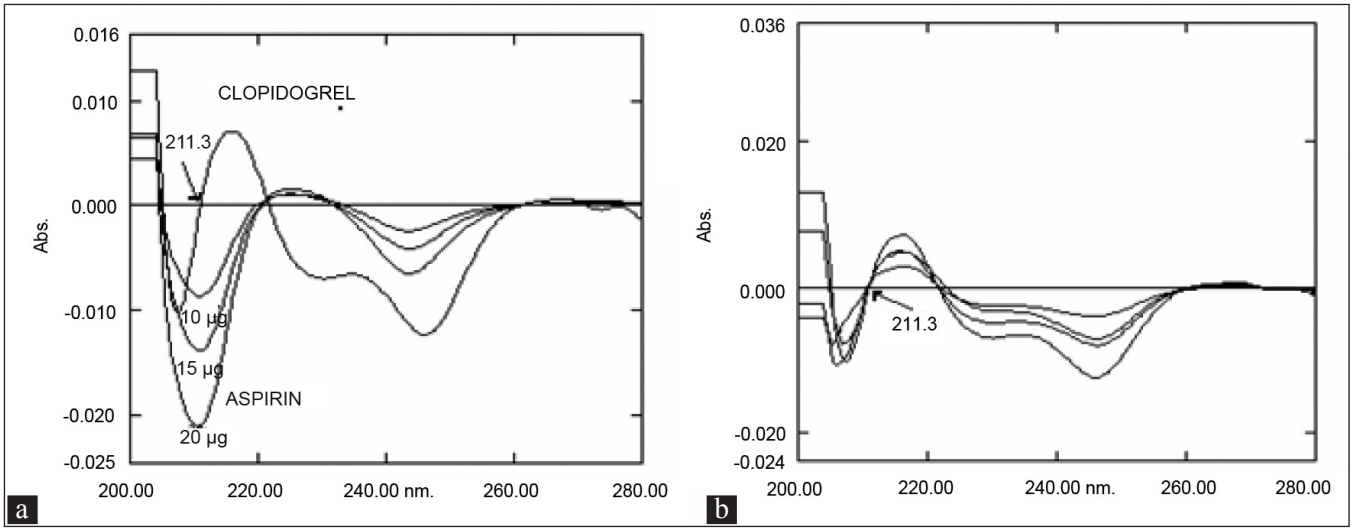

Fig. 3: First derivative spectra
(a) First derivative spectra for CPS (10 µg/ml) and ASP (10, 15, 20 µg/ml); (b) First derivative spectra for CPS (10, 15, 20 µg/ml) and ASP (10 µg/ml)

TABLE 1: STATISTICAL DATA OF CALIBRATION CURVE

Parameters	CPS	ASP
Wavelength (nm)	232.5	211.3
Beer's law limit	5-25	5-25
Regression equation*	Y= 0.0006x-0.0015	Y=0.0013-0.0042
Correlation coefficient	0.9862	0.9914
LOD (µg/ml)	2	5
LOQ (µg/ml)	5	10

y=mx+c; where x is the concentration of drug in µg/ml, y is the amplitude at specified wavelength, m is the slope and c is the intercept

TABLE 2: RECOVERY STUDY DATA

Level of standard addition (%)	% Recovery± SD*	
	CPS	ASP
80	99.68±0.2097	99.39±0.7425
100	99.77±0.3842	100.18±0.6286
120	99.72±0.8015	100.08±0.5211

*Mean of three determinations, SD is standard deviation

TABLE 3: RESULT OF VALIDATION STUDIES OF PROPOSED METHOD

Parameters	CPS	ASP
Linearity	±20 % of test conc	±20 % of test conc
Precision (% Label Claim±SD, n=5)	100.66±0.5211	98.55±0.152
Ruggedness (% Label Claim, n=3)		
Intraday	100.23	98.69
Interday	100.52	99.23
Different Analyst	99.92	99.15
Specificity	Specific	Specific

SD is standard deviation

TABLE 4: RESULT OF ANALYSIS OF COMMERCIAL FORMULATIONS

Drug	% Label claim *(mg)	±SD*
CPS	100.09	0.5799
ASP	99.87	0.2317

*Mean of three determinations. SD is standard deviation

diluted with 0.1N HCl to obtain concentration range of 2-30 µg/ml. For all solutions the derivative spectra were obtained over 200 to 400 nm range. At 232.5 nm there were well developed first order derivative absorption spectra for varying concentrations of CPS for its determination (fig. 2a) and no ASP interferences was observed as D_1=0 (fig. 2b). So any change in ASP concentration has no effect on quantitative determination of CPS.

To determine ASP the first order derivative spectra were used by making measurements at 211.3 nm (fig. 3a) at which D_1=0 for CPS. No CPS interferences were found even at different concentrations (fig. 3b) for quantitative determination of ASP.

The calibration curves were constructed by plotting drug concentration versus the absorbance values of first derivative spectrum (D_1) 232.5 nm for CPS and 211.3 nm for ASP. Statistical data for calibration curves is depicted in (Table 1) The concentration of individual drugs present in the mixture was determined from the calibration curves in quantitation mode.

Twenty tablets of brand (Clopivas AP Cipla Ltd, Mumbai) containing 75 mg of CPS and 75 mg of ASP per tablet were weighed accurately, average weight determined and finely powdered. The powder equivalent to 10 mg of CPS and 10 mg of ASP was weighed accurately and transferred to 100 ml volumetric flask. Twenty milliliters of methanol was added to the flask and sonicated for 20 min. The solution was filtered through Whatman filter paper (No. 41) and the volume was adjusted up to the mark with methanol. This solution is expected to contain 100 µg/ml CPS and 100 µg/ml ASP. One millilitre of this solution was transferred to a 10 ml volumetric flask and volume was made up the mark with 0.1N HCl to obtain final concentration of CPS (10 µg/ml) and ASP (10 µg/ml). The concentration of both CPS and ASP were determined by measuring the absorbance at 232.5 nm and 211.3 nm in first order spectrum mode and the results of tablets analysis were calculated from the calibration curve in quantitation mode.

The method was validated statistically as per ICH/ USP16 guidelines for all the parameters like accuracy, linearity, precision, ruggedness and specificity. Accuracy of the method was ascertained on the basis of recovery studies, carried out by standard addition method in which pre-analyzed samples were taken and standard drug was added at three different levels. (80%, 100% and 120% of the test concentration). The % recovery±SD lies in the range of 99.68±0.2097 to 99.77±0.3842 for CPS and 99.39±0.7425 to 100.18±0.6286 for ASP (Table 2). The linearity of the method was established from the first derivative spectra by measurement of absorbance of standard solutions containing varying concentrations of each compound in the presence of constant concentrations of other one. Linearity was constructed in the range of 5-25 µg/ml (r^2<1). CPS and ASP in tablets were found to be linear in the range ±20 % of test conc. Precision was studied by analyzing five replicates of sample solutions and concentrations were calculated. Ruggedness was established by carrying out experiment at different conditions like intra-day, inter-

day and by different analyst. Specificity of the method was ascertained by analysing standard drug and sample. There was no interference of the excipients present in the formulation. By observing validation parameter (Table 3) the method described was found to be specific, accurate, precise and economical and can be successfully applied to analyze commercially available tablets containing CPS and ASP. The results obtained are in good agreement with the labeled content, summarized in (Table 4).

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