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Quantitative Analysis for Clopidogrel Bisulphate and Aspirin by Second Derivative Spectrophotometric Method in Pharmaceutical preparation

Madhuri D.Game* and D. M. Sakarkar

[1]Vidyabharati College of Pharmacy, C.K.Naidu road, Camp, Amravati – 444602,MS, India

[2]Sudhakarrao Naik Institute of Pharmacy, Pusad - 445204 ,MS,India

**Corres. author: game_madhuri@yahoo.co.in*
Mobile no: 09860613039

Abstract: A simple, accurate and precise spectrophotometric method was developed for simultaneous estimation of clopidogrel bisulphate and aspirin by employing second order derivative zero crossing method .The second order derivative absorption spectra at 254.0 nm (zero cross point of aspirin) were used for clopidogrel bisulphate and 216.0 nm (zero cross point of clopidogrel bisulphate) were used for aspirin.No interferences were found between both determined constituents and those of matrix. A good accuracy and precision of simultaneous determination of clopidogrel bisulphate and aspirin were confirmed by statistical analysis. The recovery of individual constituents under established conditions is very high and ranges from 99.68 % to 100.18 %. Linearity is maintained within a wide concentration range from 5.0 $\mu g.mL^{-1}$ to 30.0 $\mu g.mL^{-1}$ for clopidogrel bisulphate and aspirin both. The detection limit is 2.0 $\mu g.mL^{-1}$ and 5.0 $\mu g.mL^{-1}$ and the corresponding quantitation limits are same that is 5.0 $\mu g.mL^{-1}$ for clopidogrel bisulphate and aspirin respectively
Keywords: clopidogrel bisulphate, aspirin, drug analysis, derivative ,spectrophotometry.

Introduction

Clopidogrel bisulphate (CPS) is methyl-2-chlorophenyl-(4,5,6,7-tertrahydrothieno[3,2-]pyridine-5yl)acetate bisulphate and aspirin (ASP)is 2-acetoxy benzoic acid used in the treatment of cardiovascular diseases. Clopidogrel is used as platelet inhibitor and aspirin as a cyclooxygenaseinhibitor[1,2]. Monographs of various pharmacopoeias describe the assay of aspirin. Aspirin is a well studied drug, official in all the pharmacopoeias where as clopidogrel is not official in any of the pharmacopoeias. Literature review reveals few HPLC methods for estimation of both the drug as single component in different dosage forms and few methods have been reported for analysis of these drugs in multicomponent dosage forms[3-10]. The objective of present communication was to develop simple, rapid and precise method for concurrent estimation of CPS and ASP in combined dosage form. To improve therapy a number of drugs are given in combination .As these drugs have similar physicochemical properties these arise difficulty in identification and quantitation. Before analysis prior separation is necessary in chromatography, electrophoresis etc. Derivative spectrophotometry is now becoming a practical analytical method because of quicker and more accurate quantitation of multicomponent mixture without prior separation. The zero crossing technique has been found practical application more recently. Several papers have been published on zero crossing technique using various order of derivatives. Derivative spectrophotometric method provides a greater selectivity than common spectrophotometric methods and offers a powerful approach for resolution of band overlapping and quantitative analysis of multicomponent mixtures. In this paper a new derivative spectrophotometric method

for simultaneous determination of CPS and ASP is presented. An attempt was made to find suitable derivative and wavelength for quantitative analysis at which there was no interference of other constituents.

Materials and Method

Pure drugs of CPS and ASP were obtained as gift sample from Lupin Laboratories SIDCO industrial Complex Jammu. A.R. grade methanol from Qualigens, Mumbai was used as solvent for preparing solutions. The solution of 0.1 N 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 (2nm) and 1 cm matched quartz cells was used for all the spectral measurements. Standard stock solutions (100 $\mu g.mL^{-1}$) of CPS and ASP were prepared by separately dissolving 10mg each of CPS and ASP in 100ml methanol. Suitable aliquot of standard stock solutions were diluted with 0.1 N HCl. to obtain solutions of CPS (10 $\mu g.mL^{-1}$) and ASP (10 $\mu g.mL^{-1}$). 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. Second order derivative spectra were selected for analysis of both the drugs. From the overlain spectra of both the drugs (figure.1) wavelengths selected for quantitation were 216.0 nm (zero cross point of clopidogrel bisulphate) for ASP and 254.0 nm (zero. cross point of aspirin) for clopidogrel bisulphate. The standard stock solutions of CPS and ASP were diluted with 0.1 N HCl to obtain concentration range of 2-30 $\mu g.mL^{-1}$ .For all solutions the derivative spectra were obtained over 200 to 400 nm.range.

At 216.0 nm. there were well developed second order derivative absorption spectra for varying concentrations (Figure 2) for determination of ASP. At 216.0 nm no CPS. interferences were observed as D2 = 0.for CPS.So any change in CPS concentration has no effect on quantitative determination of ASP. To determine CPS. the second order derivative spectra were used by making measurements at 254.0 nm (Figure3) at which $D_2 = 0$ for ASP. No ASP interferences were found even at different concentrations.The calibration curves were constructed by plotting drug concentration versus the absorbance values of second derivative spectrum (D_2) 254.0 nm for CPS and 216.0 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.

Application of the Proposed Methods for the determination of CLO and ASP in Tablets

In order to see the feasibility of proposed method for simultaneous estimation of CPS and ASP in marketed pharmaceutical formulations, the method was first tried for estimation of both drugs in standard laboratory mixtures, obtained by mixing the aliquot portions of individual stock solutions to get final concentration of CPS (10 $\mu g.mL^{-1}$) and ASP (10 $\mu g.mL^{-1}$) respectively. The results were found satisfactory and hence this method was further applied to marketed preparations. 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. An accurately weighed powdered sample equivalent to average weight of tablet was transferred to a beaker, dissolved in methanol filtered through Whatman filter paper (No. 41) in 100 ml volumetric flask and the volume was made upto the mark with methanol. This solution is expected to contain 100 $\mu g.mL^{-1}$ CPS and 100 $\mu g.mL^{-1}$ ASP, necessary dilutions were made with 0.1N HCl. to obtain final concentration of CLO (10 $\mu g.mL^{-1}$) and ASP (10 $\mu g.mL^{-1}$) respectively. The concentration of both CPS and ASP were determined by measuring the absorbance of sample at 254.0 nm and 216.0 nm in second order spectrum mode and the results of tablets analysis were calculated from the calibration curve in quantitation mode.

Validation:

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). Result of recovery studies and percentage recovery were found to be satisfactory and are reported in Table 2 The linearity of the method was established from the second 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 2-30 $\mu g.mL^{-1}$ ($r^2<1$). 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 of assay were in good agreement with the labeled content, summarized in (Table 4).

TABLE 1- STATISTICAL DATA OF CALIBRATION CURVES OF CLOPIDOGREL BISULPHATE AND ASPIRIN USING SECOND ORDER DERIVATIVE SPECTRA.

Parameters	Clopidogrel Bisulphate	Aspirin
Wavelength (nm)	254.0	216.0
Linearity (µg/ml)	2-30	5-30
Regression equation *	Y = 0.0003x - 0.0008	Y = 0.0003 – 0.0008
Correlation coefficient	0.9839	0.9813
LOD (µg/ml)	2	5
LOQ (µg/ml)	5	5

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

TABLE 2: RECOVERY STATUS DATA

Level of standard addition (%)	% Recovery*± SD*	
	CPS	ASP
80	99.75 ± 0.3265	99.68 ± 0.2097
100	99.85 ± 0.2599	100.05 ± 0.5233
120	100.18 ± 0.6286	99.79 ± 0.3305

*Mean of three determinations, SD is standard deviation

TABLE 3: RESULT OF VALIDATION STUDIES OF PROPOSED METHOD

Parameters	Clopidogrel Bisulphate	Aspirin
Linearity	± 20 % of test conc.	± 20 % of test conc.
Precision (% Label Claim± SD,n=5)	105.66 ± 0.4201	99.85 ± 0.252
Ruggedness (% Label Claim n=3)		
Intraday	100.23	99.99
Interday	100.52	98.26
Different Analyst	99.92	99.15
Specificity	Specific	Specific

(n=5), (n=3) results are mean of three determinations, SD is standard deviation

TABLE 4- RESULT OF ANALYSIS OF COMMERCIAL FORMULATIONS.

Drug	% Label claim *(mg)	±SD*
Clopidogrel bisulphate	100.09	0.5799
Aspirin	99.87	0.2317

*Mean of three determinations.

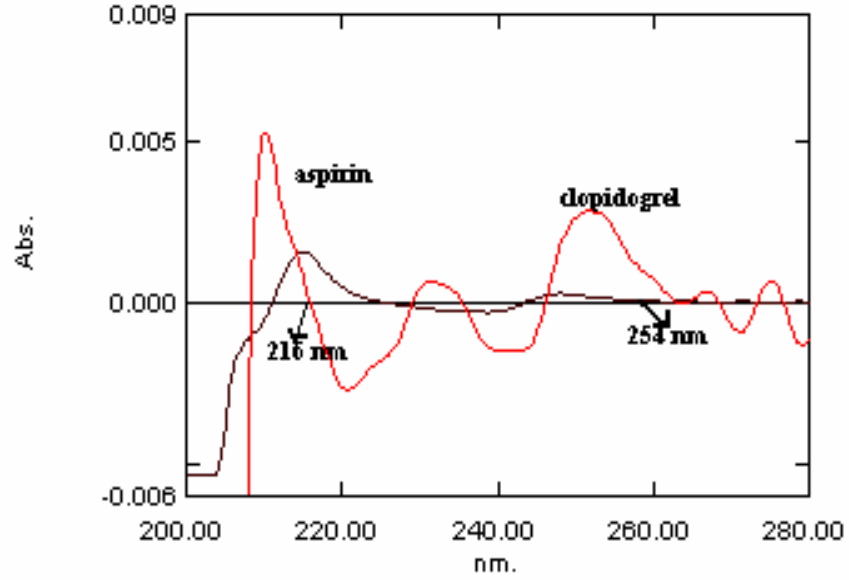

Figure 1:Overlain second order derivative UV spectra for clopidogrel and aspirin

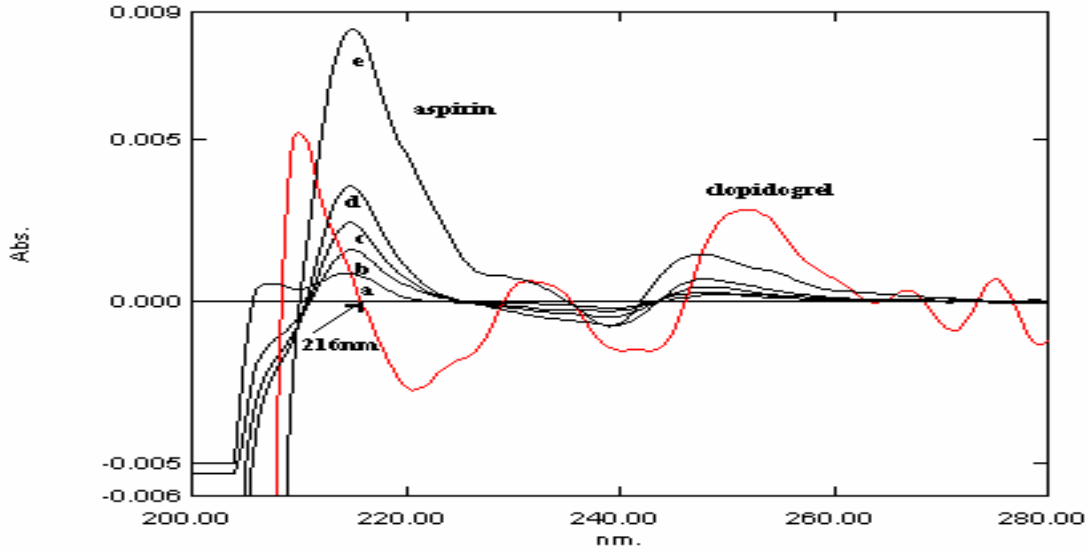

Figure 2- second order UV derivative spectra for clopidogrel and aspirin of concentrations (a=5.0 µg.mL^{-1} ,b= 10 µg.mL^{-1},c=15 µg.mL^{-1},d=20 µg.mL^{-1} ,e=30 µg.mL^{-1})

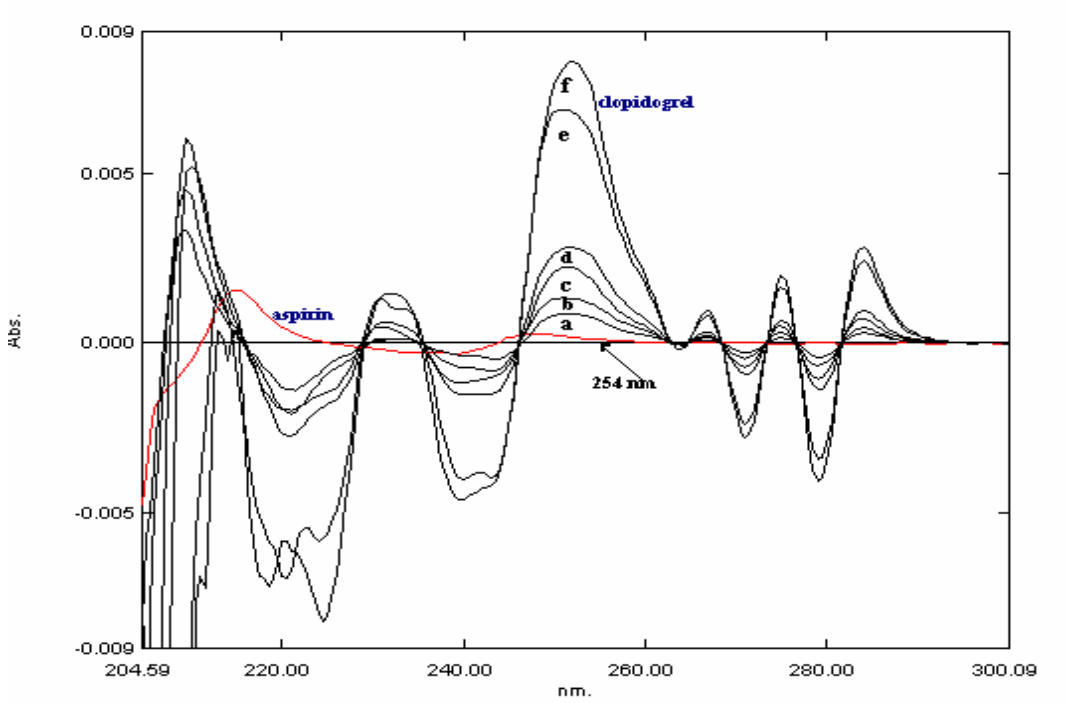

Figure 3- Second order UV derivative spectra for aspirin and clopidogrel of concentrations (a =5.0 µg.mL^{-1} b= 10 µg.mL^{-1},c =15 µg.mL^{-1} ,d =20 µg.mL^{-1} e =30 µg.mL^{-},f =35 µg.mL^{-1})

Results and Discussion

Due to high sensitivity and simple sample preparation, spectrophotometric methods have obvious advantages over sophisticated instrumental analysis such as HPLC. The method described was found to be simple ,accurate, economical and rapid for routine simultaneous estimation CPS and ASP and can be used for undergraduate studies. Hence, simple and economical instrumental methods always have a role in pharmaceutical analysis.

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