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Research Article

H-POINT STANDARD ADDITION METHOD FOR THE SIMULTANEOUS DETERMINATION OF CLOPIDOGREL BISULFATE IN A MIXTURE WITH ASPIRIN

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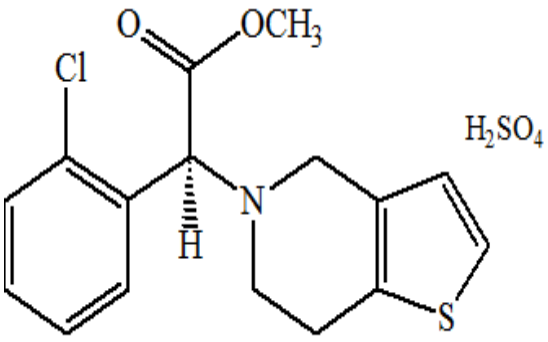
ABSTRACT

H-point method was developed for the quantitative determination of clopidogrel bisulfate (CLPB). HPSAM showed suitable abilities to resolve accurately overlapped absorption spectra of the compounds. So a simultaneous determination of CLPB in its binary mixture with aspirin (ASP) was carried out. They can be determined simultaneously with the concentration ratios 1:4 to 4:1 in their mixed sample. The calibration curves of CLPB and ASP were constructed at the maximum wavelength of each (266 and 277 nm for CLPB and 276 and 281 nm for ASP).The method showed good precision and high recovery values for the determination of the two drugs in presence of each other.

Keywords: Clopidogrel bisulfate, Aspirin, H-point standard addition, Spectrophotometry, Aspirin.

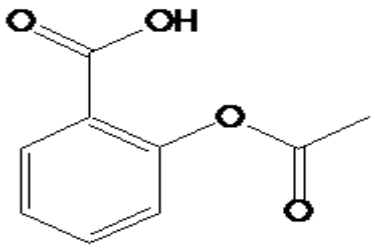
INTRODUCTION

(2S)-2-(2-chlorophenyl)-2-(6,7-dihydro-4H-thieno[3,2-c]pyridine-5-yl)acetate is known as clopidogrel bisulfate ($C_{16}H_{16}ClNO_2S•H_2SO_4$, Mol wt = 419.9 g mol^{-1}),Scheme 1[1].It is a white to off-white powder, practically insoluble in water at neutral pH but freely soluble at pH 1, dissolves freely in methanol, sparingly in methylene chloride and practically insoluble in ethyl ether[1].



Scheme. 1: Structural formula of clopidogrel bisulphate

It acts by direct inhibition of ADP binding to its receptor and of subsequent ADP mediated activation of glycoprotein GPIIb/IIIa complex. It is used as an effective drug for reducing the incidence of ischemic strokes, heart attacks or claudicating due to vascular diseases such as atherosclerosis[2]. Clopidogrel in combination with aspirin (Scheme 2) significantly reduced collagen-induced platelet aggregation. Clopidogrel (CLPB) is an oral, thienopyridineclass antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease[3].



Scheme. 2: Structural formula of aspirin

Literature survey reveals different types of assay methods for the determination of CLPB as single component in different dosage forms and few methods have been reported for analysis of these drugs in multicomponent dosage forms. It includesspectrophotometric[4-10], chemometric[11], capillary electrophoresis [12,13], HPLC[14,22], HPTLC[15,23,24], and LC[25-27] methods. The aim of the present work is to develop simple, rapid, reproducible, inexpensive procedure suitable for the routine quality control analysis of the investigated drug in its binary mixture with aspirin. Spectrophotometric method still belongs to the most frequently used analytical techniques in pharmaceutical analysis, which gives practical and significant economic advantages over other methods.

MATERIALS AND METHODS

Apparatus

All the spectral measurements were carried out using a Jenway 6105 UV/V single beam spectrophotometer equipped with glass or quartz cells of 1 cm optical path length and microburette (1/50). A Scientech SA 210 digital balance was used for weighing throughout the study.

MATERIAL AND REAGENTS

All chemicals and reagents used in this investigation were of the highest purity available.Bidistilled water was used throughout this study. Methanol was HPLC grade (Lab scan). Clopidogrel bisulfate (CLPB) and aspirin (ASP) bulk powders were obtained from Pfizer, Egypt, Plavix (75 mg/tablet) was manufactured by Sanofi Aventis, France and Borgavix (75 mg/tablet) was obtained from Borg Pharmaceutical Industries.Colsprin were prepared in our lab (authentic sample, 75 mg CLPB + 75 mg ASP/tablet).

Solutions

1.0×10^{-3}mol L^{-1} of CLPB and ASP stock solutions were prepared in methanol.

Procedure for tablets

As a result of the non-existence of Closprin (tablet formulation; containing 75.0 mg CLPB and 75 mg ASP/tablet) in local market, authenticmixture imitating this product was prepared in the laboratory.

General procedures

A suitable volume of 1.0×10^{-3}mol L^{-1} CLPB or ASP was transferred in a spectrophotometric cell (1 cm bath length). The absorbance was measured in the range 250-300 nm against methanol as blank. Individual calibration graphs were constructed by transferring different volumes of 1.0×10^{-3} mol L^{-1} CLPB or ASPinto 10 mL measuring flask and completed to the mark by methanol. Synthetic samples containing different concentration ratios of CLPB and ASP were prepared. HPSAM was performed by measuring the absorbance at the selected wavelength when standards of ASP or CLPB were added, respectively.

RESULTS AND DISCUSSION

HPSAM is a modification of the standard addition method, permits both proportional and constant errors produced by the matrix of the sample to be corrected directly. The fundamentals of HPSAM were outlined by Bosch Reig and Campins Falco in 1988[28]. It was proposed in order to obtain an unbiased analyte concentration when both analyte and interferent are present in a sample. It was established for resolving spectra of two analytes with strongly overlapping spectra[28,29]. This method is based on the dual wavelength spectrophotometry and the standard addition method. The requirements for application of this method is that only to work at two wavelengths where the analytical signal due to one of the species (Y) is constant and for another one (X) as different as possible. By plotting the analytical signal verses added X concentration, two straight lines are obtained that have a common point with coordinates H ($-C_H$, A_H). Where $-C_H$ is the unknown X concentration and A_H is the analytical signal due to Y species. The method can be applied to the resolution of mixtures of two components with extensively or even completely overlapping (in the latter case with coincident peak maxima but different spectral features). Absorbance increments as analytical signals were used when only the X concentration was required[30]. The method has been applied to eliminate the blank bias error due to the use of absorbent blank [31,32], in liquid chromatography[33] and to the analysis of kinetic data[34,35], with time as an additional variable.

Absorption spectra

It can be used for an unknown sample containing an analyte X and an interferent Y. In this special system, either CLPB or ASP can be considered as the analyte and the other one as interferent. The determination of concentration of X under these conditions requires the selection of two wavelengths λ_1 and λ_2, at which the interfering species, Y, should have the same absorbance[21,36]. Then, known amounts of X are successively added

to the mixture and the resulting absorbance is measured at the two wavelengths. A suitable volume of 1.0×10^{-3} mol L^{-1} CLPB or ASP was transferred in a spectrophotometric cell (1 cm bath length). The absorbance was measured in the range 250-300 nm against methanol as blank. The selected wavelengths are 266 and 277 nm for CLPB and 276 and 281 nm for ASP as seen in Fig.1.

Individual calibration

At the two selected wavelengths, the signal of Y species must remain equal, even if the concentrations of X species are changed, and the analytical signals of the mixture composed of X and Y should be equal to the sum of the individual signals of the two species. In addition, the slope difference of the two straight lines obtained at λ_1 and λ_2 must be as large as possible in order to get good accuracy.

Individual calibration graphs were constructed with several points (Fig. 2) at 266 and 277 nm for CLPB and 276 and 281 nm for ASP, as absorbance versus drug concentration up to 300.0and 75.0 µg mL^{-1} for CLPB and ASP, respectively, evaluated by linear regression. The intercepts on the ordinates were negligible in the graphs.

Applying H-point standard addition method (HPSAM)

When CLPB is selected as the analyte (X), it is possible to select several pairs of wavelengths where they present the same absorbance for ASP. After this, the selected wavelength pairs were sorted following the criteria to give the high values for the difference of the calibration slopes. The equation that is used for calculation of C_H can be seen as a ratio between absorbance increment (ΔA) and a slope increment (ΔM). ΔMdepends on the analyte absorption characteristics, and also ΔA depends on the sample analyteconcentration. As shown previously by Campins-Falco et al.[29], the higher the value for the slope increment the smaller the error for the analyte concentration. The concentration of Y species was calculated in each test solution by employing the calibration line obtained from ordinate values of H-point (A_H) for standard binary solutions. Employing the proposed procedure on several synthetic samples showed that, C_H(concentration of analyte) is independent of the concentration of interference and so A_H(absorbance corresponding to interference concentration) also is independent of the analyte concentration.

Plots of H-point standard addition method for addition of ASP as standard at wavelengths 266 and 277 nm for a mixture of 54.05 µg mL^{-1} of ASP and 83.98 µg mL^{-1} of CLPB and addition of CLP as standard at wavelengths 276 and 281 nm for a mixture of 54.05 µg mL^{-1} of ASP and 41.99 µg mL^{-1} of CLPB, Fig.3.

Several synthetic mixtures with different concentrations of CLPB and ASP were analyzed by using the suggested method. As can be seen from Table 1, the accuracy and precision of the results are all satisfactory, when the concentration ratio of CLP and ASP vary from 4:1 to 1:4.

CONCLUSION

H-point standard additions method has been described for the determination of clopidogrel bisulfate. HPSAM method is recommended as a very suitable choice to resolve accurately overlapped absorption spectra of the compounds and to remove matrix effects easily. Therefore, this method can be used for resolving binary mixtures of CLPB and ASP. Spectrophotometric method has advantageous over many of the reported methods due to their sensitivity, high percentage of recovery, wide application range, and they do not need expensive sophisticated apparatus. Therefore, the methods are practical and valuable for routine application in quality control laboratories for analysis of CLPB in its binary mixture with aspirin.

Table 1: Analysis of CLPB-ASP mixtures in different concentration ratios using HPSAM

A-C equation	r^2	Taken (µg mL^{-1})		Found (µg mL^{-1})		Recovery (%)	
		CLPB	ASP	CLPB	ASP	CLPB	ASP
$A_{276} = 1.28\times10^{-3}C_i + 0.39$	0.9950	41.99	54.05	41.40	53.18	98.59	98.39
$A_{281} = 8.31\times10^{-4}$ Ci + 0.37	0.9919						
$A_{276} = 1.22\times10^{-3}$ C_i + 0.34	0.9925	83.98	36.03	82.32	36.47	98.02	101.22
$A_{281} = 8.17\times10^{-4}$ C_i + 0.31	0.9980						
$A_{266} = 4.66\times10^{-3}$ C_i + 0.38	0.9995	83.98	54.05	83.49	53.79	99.42	99.52
$A_{277} = 6.39\times10^{-3}$ C_i + 0.45	0.9969						
$A_{266} = 4.38\times10^{-3}$ C_i + 0.32	0.9998	41.99	54.05	44.31	55.32	105.53	102.35
$A_{277} = 5.99\times10^{-3}$ C_i + 0.40	0.9990						
$A_{266} = 4.44\times10^{-3}$ C_i + 0.29	0.9989	83.98	36.03	91.60	35.99	109.07	99.89
$A_{277} = 6.27\times10^{-3}$ C_i + 0.36	0.9983						
$A_{266} = 4.89\times10^{-3}C_i$ + 0.34	0.9831	125.97	36.03	124.57	35.13	98.89	97.50
Closprin (authentic sample, (75 mg CLPB + 75 mg ASP)/tablet)							
$A_{277} = 6.89\times10^{-3}$ C_i + 0.40	0.9899						
$A_{266} = 4.31\times10^{-3}$ C_i + 0.24	0.9989	37.59	37.83	38.76	37.62	103.13	99.45
$A_{277} = 7.38\times10^{-3}$ C_i + 0.34	0.9986						
$A_{266} = 4.52\times10^{-3}$ C_i + 0.32	0.9998	52.61	52.97	51.93	52.30	98.71	98.74
$A_{277} = 7.48\times10^{-3}$ C_i + 0.45	0.9987						

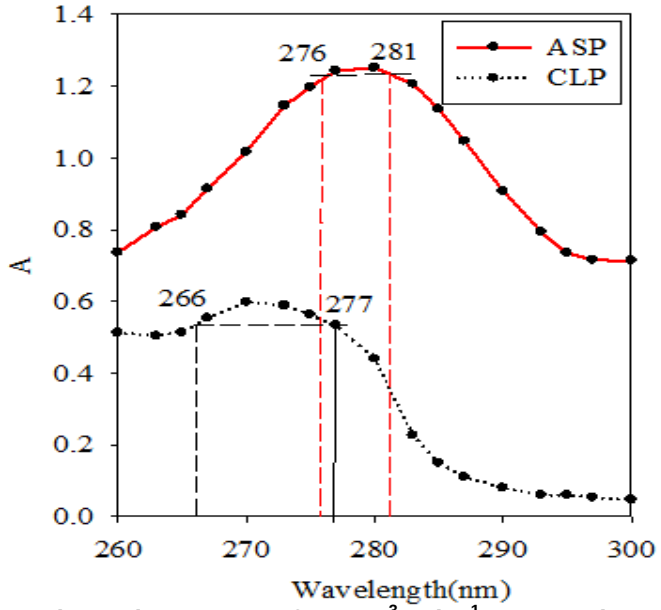


Fig. 1: Absorption spectra of 1.0×10^{-3}mol L^{-1} CLPB and ASP

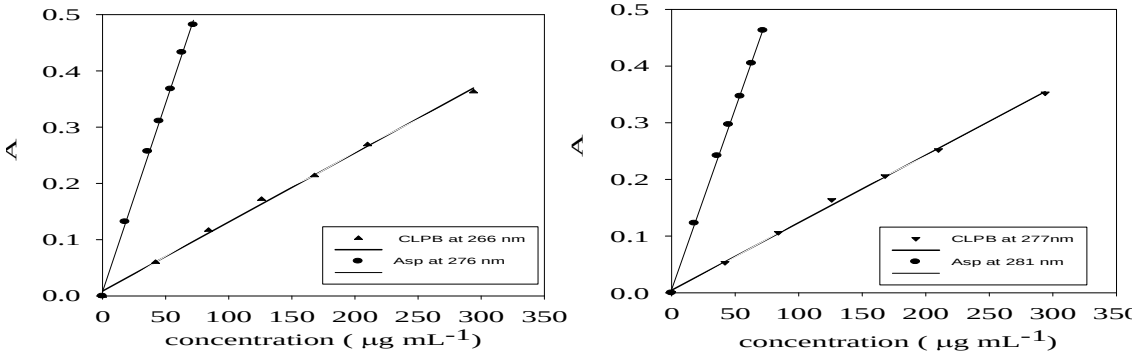


Fig. 2: Individual calibration graphs for CLP (at λ = 266, r^2 = 0.9975 and at λ = 277, r^2 = 0.9985) and ASP (at λ = 276 nm, r^2 = 0.9987 and at λ = 281 nm, r^2 = 0.9992)

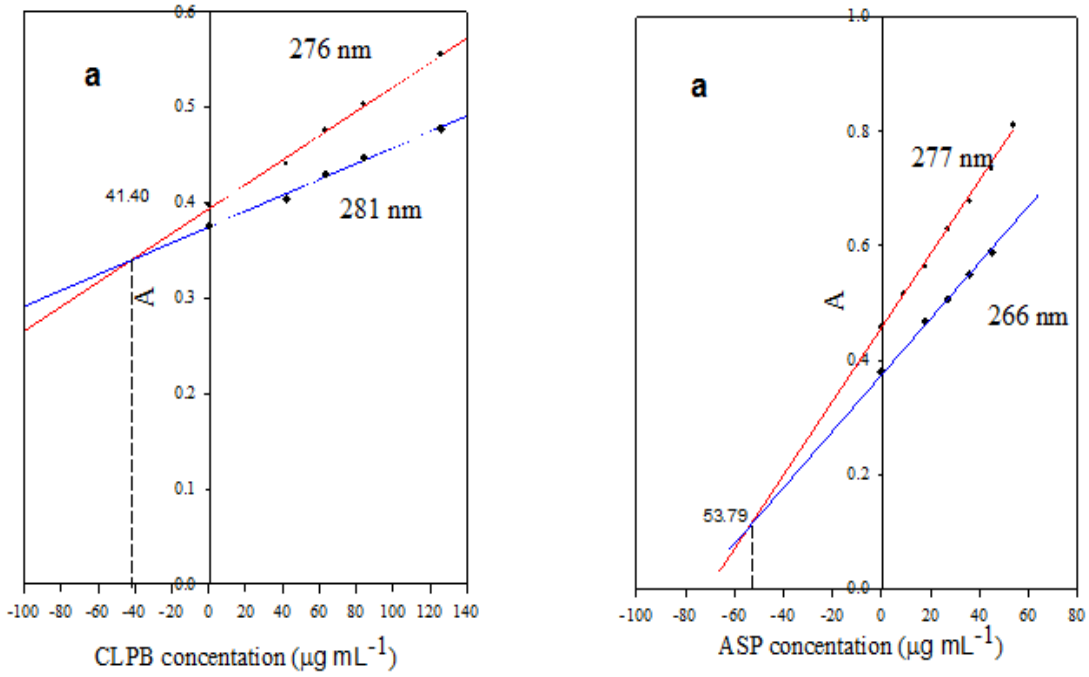


Fig. 3: Plots of HPSAM for (a) a mixture of ASP (54.047 µg mL^{-1}) and CLPB (41.990 µg mL^{-1}). and (b) a mixture of ASP (54.047 µg mL^{-1}) and CLPB (83.980 µg mL^{-1})

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