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Novel spectrophotometric methods for the determination of clopidogrel bisulphate in bulk and pharmaceutical formulations by cobalt thiocyanate and Tpooo

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ABSTRACT

Simple and sensitive two visible spectrophotometric methods M_1 and M_2 are described for the assay of clopidogrel bisulphate in pure and solid dosage forms. The method M_1 is based on the formation of coordination complex between drug (electron donor) and CTC, colored species is formed and exhibits absorption maxima at 625nm. The method M_2 is based on ion association complex formation of Tpooo with drug and exhibits absorption maxima at 485 nm. Regression analysis of Beer's-lambert plots showed good correlation in the concentration ranges (1.0-5.0 ml, 500 µg/ml) for the method M_1, (1.0-5.0 ml, 500 µg/ml) for the method M_2 respectively. The proposed methods are applied to commercial available tablets and the results are statically compared with these obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the clopidogrel bisulphate in the presence of other ingredients that are usually present in dosage forms. These methods offer the advantages of rapidity, simplicity and sensitivity and normal cost can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Key words: Clopidogrel bisulphate, Assay, Beer's law, Cobalt thiocyanate, Tpooo, Regression equation.

INTRODUCTION

Clopidogrel bisulphate (CPD) is a prodrug activated in the liver by cytochrome P450 enzymes, the action of which may be related to an ADP receptor on platelet cell membranes. It is chemically known as Methyl (+)-(S)-α-(2-chlorophenyl)-6, 7-dihydrothieno [3, 2-c] pyridine-5(4H) acetate sulfate (1:1). CPD is a new thienopyridine derivative chemically related to ticlodipine. It has been shown to prevent stroke, myocardial infarction and demonstrated clinical efficacy superior to that of aspirin[1]. The drug specifically and irreversibly inhibits the subtype of ADP receptor, which is important in activation of platelets and eventual cross-linking by the protein fibrin. The blockade of this receptor inhibits platelet aggregation by blocking activation of the pathway, thus

preventing strokes and heart attacks[2]. Activation of this receptor complex is the "final common pathway" for platelet aggregation and is important in the cross-linking of platelets by fibrin. A very few physico-chemical methods appeared in the literature for determination of CPD in pharmaceutical formulations(less). Few methods for the determination of CPD have been reported like H-NMR[3], HPLC[4], GC-MS[5], RPHPLC[6-8],HPTLC[9,10], UV[11,12], LC method[13-16] , Colorimetry[17]. But to the best of our knowledge there is no single method available for the estimation by visible spectrophotometry which is far simple and economical and less time consuming as compared to above mentioned methods. So the authors have made some attempts in developing visible spectrophotometric methods and succeeded in devoloping two methods based on the reaction between the drug and acidic dyes namely Tpooo and CTC under specific experimental conditions.

As the extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the assay of drugs, the extractive spectrophotometric acid dye technique [18] was therefore, utilized in the present work for the estimation of CPD. The present paper describes two simple and sensitive extraction visible spetrophotometric methods for the determination of CPD, based on its tendency to form nitrobenzene extractable complexes with a dye CTC belonging to chromogenic reagent (M_1), chloroform extractable ion-associates with acidic dye Tpooo belonging to azo category dye (M_2) under experimental conditions by exploiting the basic nature of the drug molecule. According to the literature, it is the first time for CPD determination in formulations by visible spectrophotometry.

The proposed methods for CPD determination have many advantages over other analytical methods due its rapidity, lower cost and environmental safety. Unlike HPLC, HPTLC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are inexpensive and available in

any analytical laboratory. The proposed methods report a new for the determination of CPD in pharmaceuticals. These methods can be extended for the routine assay of CPD formulations.

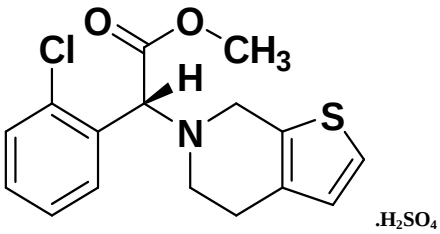

Fig. 1 chemical structure of CPD

MATERIAL AND METHODS

An Elico SL 218 Double Beam UV-Visible spectrophotometer with 1 cm matched quartz cells were used for all spectral and absorbance measurements. A systronics digital pH meter 361 was used for pH measurements. All the chemicals used were of analytical grade. Pure CPD drug was obtained as a gift sample from Dr. Reddy's laboratories Hyderabad (AP). Tablets were purchased from local market.

PREPARATION OF SOLUTIONS:

1. CTC ($2.50x10^{-1}$M) solution prepared by dissolving 7.25gm of cobalt nitrate (**BDH**) and 3.8 gm of ammonium thiocyanate(**BDM**) in100ml distilled water. Nitrobenzene (Qualigens) Solution used as it is. Buffer P^H 2.0 solution prepared by mixing 25 ml of potassium chloride solution (0.2M) and 13 ml of HCl(0.2M) and made up to 100ml ditilled water and the P^H was adjusted to 2.0.

2. TPooo (Loba: 0.5%w/v, $1.43x10^{-2}$M) solution prepared by dissolving 500mg of TPooo in 100 ml of distilled water. HCl (Qualigens: 0.1M) solution prepared by diluting 8.5 ml of con.HCl to 1000ml with distilled water.

3. Chloroform used as it is.

PREPARATION OF STANDARD DRUG SOLUTION:
The stock solution (1mg/ml) of drug was prepared by dissolving 100 mg of it in 100ml of distilled water. A portion of this stock solution was diluted stepwise with the distilled water to obtain the working standard drug solution of concentrations of 100µg/ml (M_1, M2). The prepared stock solution was stored at 4^0 C protected from light. From this stock solution, a series of standards were freshly prepared during the analysis day.

PREPARATION OF SAMPLE SOLUTION:
About twenty tablets were weighed to get the average tablet weight and pulverized. The powder equivalent to 100 mg of CPD was weighed, dispersed in 25ml of isopropyl alcohol, sonicated for 15 minutes and filtered through Whatman filter paper No.41. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

METHOD M_1:
Aliquots of standard CPD solution (1.0-5.0 ml, 500 µg/ml) were delivered into a series of 125 ml separating funnels. Then 3.0 ml of pH 2.0 buffer solution and 7.0 ml of CTC solution were added and the total volume of aqueous phase in each funnel was adjusted to 15.0 ml with distilled water. To each separating funnel, 10 ml of nitrobenzene was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated nitrobenzene layer was measured immediately at 625 nm against a similar reagent blank. The colored species was stable for 1 hr. The amount of CPD present in sample solution was calculated from its calibration graph (Fig. 4).

METHOD M_2:
Aliquots of standard drug solution (1.0-5.0 ml, 500µg/ml) were placed separately in a series of 125 ml separating funnels. A volume of 0.1 M HCl 2.0 ml and 1.5ml of Tpooo were added respectively. The total volume of aqueous phase in each separating funnel was adjusted to 10.0 ml with distilled water. Then 10 ml of chloroform was added to each separating funnel and the contents were shaken for 2 minutes and allowed to separate. The organic layer was collected through cotton plug and the absorbance was measured immediately at 485 nm against a reagent blank. Both the colored species were stable for 4 hours.

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of Tpooo and CTC reagents, 0.1M HCl, pH buffer solutions and solvent for final dilution of the colored species were studied. Tpooo and CTC were prefered for this investigation as they yield high molar absorptive values among six dyes to different chemical classes. The water immiscible solvents tested for the extraction of colored complex into organic phase include chloro benzene, dichloromethane, carbon tetra chloride, benzene, nitro benzene, n-butanol or chloroform. Chloroform and nitrobenzene was preferred for its selective extraction of colored drug-dye complex into organic layer from the aqueous phase. The stoichiometric ratio of the drug-dye was determined by the slope ratio method and was found to be 1:1 method A and 1.5:1 for method B. the optical characteristics such as Beer's law limit, sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing ¾ th of the amount of the upper Beer's law limits), regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table-1. Commercial formulations containing CPD were successfully analyzed by the proposed methods. The values obtained by the proposed and referred methods for formulations were compared statistically by the t and F test and found not to differ significantly as an additional demonstration of accuracy; recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in table-2.

CHEMISTRY OF COLORED SPECIES:
The tertiary amine (positive charge) in drug molecule in acid medium is expected to attract the oppositely charged part (negative charge) of the dye and behave as single unit being held together by electrostatic attraction as given in scheme (fig 6and7)

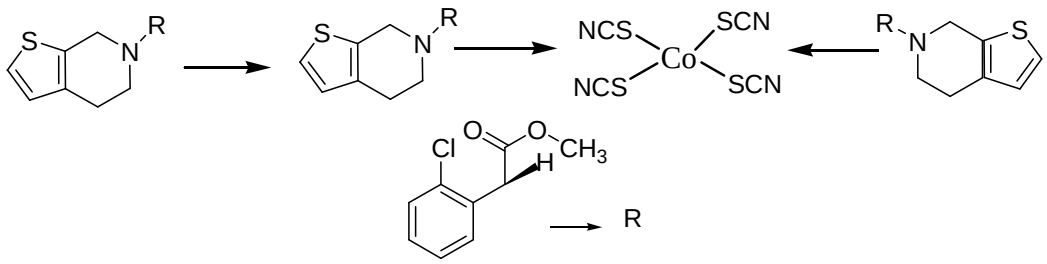

Fig 6: Probable scheme of reaction for method M_1

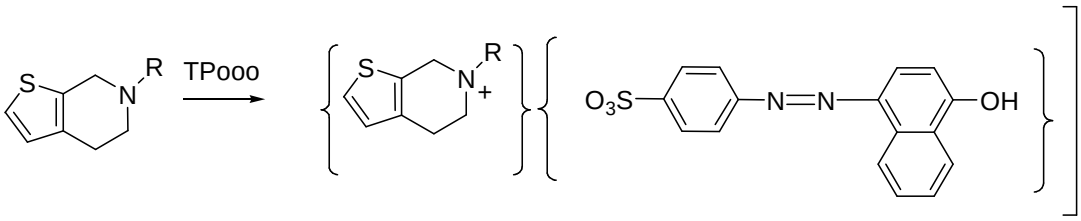

Fig 7: Probable scheme of reaction for method M_2

FIg.2: Absorption spectra of CPD with CTC system and its reagent blank

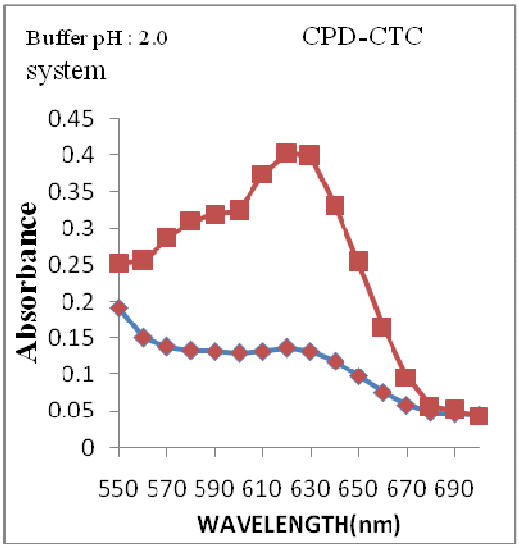

Fig. 3: Absorption spectra of CPD with Tpooo system and its reagent blank dye

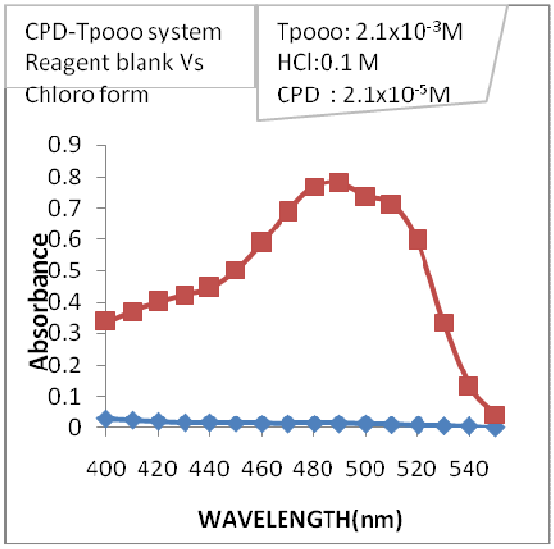

Fig.4: Beers Law plot of CPD with CTC system

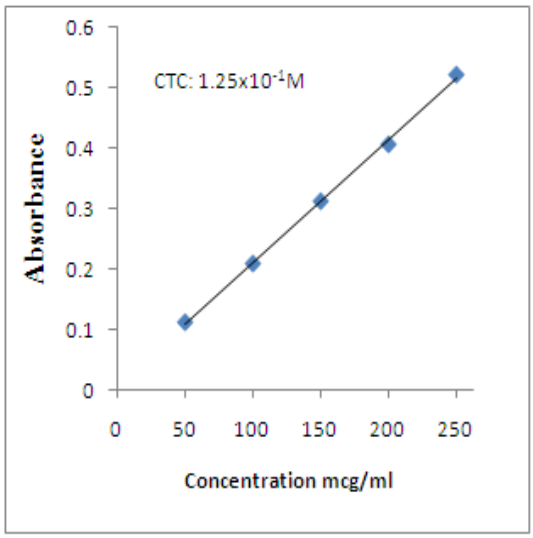

Fig. 5: Beers Law plot of CPD with Tpooo system

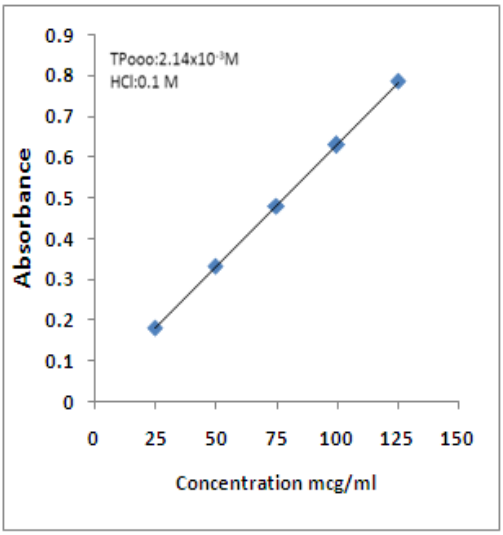

Table 1 Optical and regression characterstics, precision and accuracy of the proposed methods for CPD

PARAMETER	M_1	M_2
λ_{max} (nm)	625	485
Beer's law limits (µg/ ml)	10-40	50-250
Detection limits (µg/ ml)	$0.1848x10^4$	$0.0834x10^2$
Molar absorptivity (1 mole"1 cm"1)	$2.1362x10^5$	$6.6134x10^4$
Sandels sensitivity (µg cm^{-2} / 0.001 absorbance unit)	1.228	0.3968
Regression equation (Y = a + bC)		
Slope (b)	$6.7x10^{-3}$	$6x10^{-3}$
Standard deviation of slope (Sb)	$2.49x10^{-3}$	$4.4919x10^{-4}$
Intercept (a)	0.002	0.03
Standard deviation of intercept (Sa)	4.1291	0.0166
Standard error of estimation (Se)	0.03938	0.0408
Correlation coefficient ®	0.9989	0.9999
Relative standard deviation (%)*	0.5391	0.3033
% Range of error (Confidence limits)*		
0.05 level	0.6201	0.3488
0.01 level	1.1182	0.5469

**Y=a+bx, where Y is the absorbence and x is the concentration of CPD in µg/ml*

Table 2: Analysis of CPD in pharmaceutical formulations by proposed and reference methods

Method	*Formulations	Labeled Amount (mg)	Amount found in percentage
M_1	Batch -1 Batch -2	75 75	99.8
M_2	Batch -1 Batch -2	75 75	99.9

**Different batches from two different companies (Batch-1 Cloplet tablets of Sun Pharmaceuticals, Batch-2 Clopid tablets of Drug International Ltd.)*

CONCLUSION

A significant advantage of an extraction spectrophotometric determination is that it can be applied to the determination of individual compounds in a multi component mixture. This aspect of spectrophotometric analysis is of major interest in analytical chemistry, since, it offers distinct possibilities in assay of a particular component in a complex dosage formulation. In the present study, CPD was determined successfully as pure compound as well as a

single component in representative dosage formulations. The proposed methods applicable for the assay of drug and the advantage of wider range under Beer's law limits. The proposed extractive visible spectrophotometric methods are validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and the proposed methods report a new for the determination of CPD in pharmaceuticals. These methods can be extended for the routine assay of CPD formulations.

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