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Spectrophotometric Methods for Determination of Clopidogrel in Tablets

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Two simple, rapid, precise, highly specific and economical spectrophotometric methods have been developed for the determination of clopidogrel bisulphate in its pharmaceutical dosage forms. Method A is based on the reduction of ferric ions to ferrous ions which produce blue colour with potassium ferricyanide with absorption maximum at 760 nm. The chromogen obeyed linearity over 18-32 μg/ml. Method B is based on the hydrolysis of ester linkage of drug into acid form by heating with sulphuric acid. This acid form of drug has absorption maximum at 217 nm. Beer's law is obeyed in the concentration range of 4-18 μg/ml.

Clopidogrel hydrogen sulphate [S(α)-(2-chlorophenyl)-6,7-dihydrothieno(3,2-c)pyridine-5(4H)-acetic acid methyl ester], is a new thienopyridine derivative chemically related to ticlopidine[1-2]. It prevents ischaemic stroke, myocardial infarction and vascular disease and is indicated for the reduction of athesclerotic events and demonstrated clinical efficacy superior to that of aspirin, in a large phase 3 trial[3-4]. Only HPLC methods are reported for estimation of clopidogrel bisulphate in formulation[5] and its metabolite in plasma and serum[6]. As no spectrophotometric method is reported in the literature, and more over as clopidogrel bisulphate as such gives no absorption maximum in workable UV range, therefore this work was undertaken.

A GBC Cintra 10 UV/Vis spectrophotometer with 10 mm matched quartz cells was used for experiments. The chemicals used were of analytical grade. Ammonium ferric sulphate (CDH, Mumbai, 0.17 M in 0.1 N H2SO4), potassium ferricyanide (CDH, Mumbai, 0.17 M in distilled water) and sulphuric acid (Qualigens, Mumbai, 1 N) were used. The commercially available tablets of clopidogrel bisulphate used for estimation were procured from a local pharmacy store. Clopidogrel bisulphate (analysed sample) as provided by Dr. Reddy's Laboratories was used as such without further purification.

A solution of clopidogrel bisulphate was prepared by dissolving 10 mg (accurately weighed) of standard clopidogrel bisulphate in 10 ml of methanol. This stock solution was further suitably diluted to get a working standard solution (A) of 100 μg/ml for colorimetric method. (Method A). Similarly working standard solution (B) was separately prepared by heating appropriate volume of stock solution with 1 ml H_2SO_4.(1 N) for 30 min (Method B).

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In method A, aliquots (2, 2.2, 2.4.....3.2 ml) of working standard solution, were transferred into a series of 10 ml stoppered volumetric flasks. To that, 0.3 ml ammonium ferric sulphate and 1.0 ml potassium ferricyanide solutions were successively added, followed by 2 ml distilled water .The volumetric flasks were kept aside for 30-35 min at room temperature. The volumes were then made with distilled water. The absorbance of the blue coloured solutions formed was measured at 760 nm against the reagent blank, and the calibration curve plotted.

In method B, aliquots (0.4, 0.6, 0.8 .....1.8 ml) of working standard solution B were taken in a series of stoppered volumetric flasks, and volumes were made up with distilled water. The absorbances were taken at 217 nm against a reagent blank and calibration curve plotted.

For the present work three commercial brands of Clopidogrel tablets of 75 mg strength (Orawis, Cadila, Noklot, Zydus Medicus and Deplate, Torrent) were taken. Average weight of twenty tablets was determined and these were then finely powdered. The powdered amount equivalent to 100 mg (accurately weighed) was extracted with 4 successive 20 ml portions of methanol, filtered and the volume made up to 100 ml with methanol. Working standard solutions (A and B) were made by the procedures as mentioned above. These were then suitably diluted and the absorbances were measured accordingly to give the concentrations of the drug.

In the present work, two methods have been developed for the estimation of clopidogrel bisulphate from its pharmaceutical dosage forms. The first one is a colorimetric method, which involves the reduction of ferric ions to ferrous ions, which further reacts with potassium ferricyanide to produce the blue coloured ferro-ferricyanide[7]. The second method involves cleavage of ester linkage of drug and its conversion to acid form, upon heating with sulphuric acid. This approach had to be adopted since clopidogrel bisulphate as such gives absorption maximum in methanol at 202.9 nm, but this can not be used for estimation, since the cut off wavelength of methanol is 210 nm in UV[8]. After a systematic study, the optimum parameters found for maximum colour development (method A) were incorporated into the estimation procedure above. The optical characteristics such as absorption maxima, Beer's law limit, Correlation Coefficient(r), slope (m), intercept (c), molar absorptivity, Sandell's sensitivity were calculated and the results are incorporated in Table 1. The molar absorptivity and Sandell's sensitivity show that the methods are sensitive. The proposed methods were applied for the analysis of drug in tablets. The

TABLE 1: OPTICAL CHARACTERISTICS

Parameter	Method A	Method B
λ_{max} (nm)	760	217
Beer's Law Limits (µg/ml)	18-32	4-18
Molar absorptivity (l/mole/cm)*	$7.305x10^3$	$9.429x10^3$
Sandell's Sensitivity (µg/cmx.001 Absorbance unit)*	0.0440	0.0341
Regression equation (y=mx+c)		
Slope (m)	0.0289	0.0298
Intercept (c)	0.1475	0.004
Correlation coefficient (r)	0.9991	0.9994

*Average of eight determinations.

TABLE: 2 RESULTS OF ANALYSIS OF TABLETS

Method	Tablet	Amount (mg/tab)	Amount found* (mg/tab)	SD*	%RSD*	SE*	't' cal*	't' theo
A	ORAWIS	75	75.07±0.23	0.2983	0.3970	0.1218	0.5853	2.571
	NOKLOT	75	74.86±0.12	0.1586	0.2110	0.0647	2 .1313	
	DEPLATT	75	74.94±0.07	0.0920	0.1231	0.0375	1.3813	
B	ORAWIS	75	74.57±0.39	0.4891	0.6558	0.1997	2.1430	2.571
	NOKLOT	75	74.34±0.55	0.6968	0.9370	0.2845	2.2956	
	DEPLATT	75	74.55±0.36	0.4515	0.6055	0.1843	2.4112	

*Average of six determinations. Theoretical 't' values at 95% confidence level for (n-1) degrees of freedom 't' (0.05,5)=2.571. SD is standard deviation, % RSD is percent relative standard deviation and SE is the standard error.

results of analysis of commercial formulations significantly showed low values for standard deviation, standard error and coefficient of variation and thus show the precision of the methods. These values in each instance are compared with the theoretical value of 100 percent by means of unpaired students 't' test. (Table 2). As the calculated 't' values were less than theoretical 't' values, it is concluded that the results of analysis were in good agreement for each tablet. To test the accuracy and reproducibility of the proposed method, recovery experiments were performed by adding known amount of pure drug to previously analyzed samples, and these samples were reanalyzed by the proposed methods. The percentage recovery was close to 100% for both the methods. The results are summarized in the Table 3. The reproducibility, repeatability and accuracy of these methods were found to be good, which is evidenced by low standard deviation. The percent recovery obtained indicates non-interference from the excipients used in the formulations. Thus it can be concluded that the methods developed in the present investigation are simple, sensitive, accurate and precise. Hence these can be successfully applied in the estimation of clopidogrel in dosage forms.

TABLE 3: RECOVERY STUDIES

Method	Tablets	% Recovery ±S.D*.
A	ORAWIS	97.97±0.91
	NOKLOT	100.5±0.94
	DEPLATT	102.6±0.96
B	ORAWIS	102.8±0.96
	NOKLOT	101.9±0.97
	DEPLATT	98.35±0.93

*average of six determinations. SD is the standard deviation

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Microwave Assisted Synthesis, AntiHIV, and AntiYFV Activities of Schiff Bases of N-Hydroxy-N^1-Aminoguanidine Tosylate

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The microwave-assisted syntheses of N-hydroxy-N1-aminoguanidines (S1-S8) starting from thiosemicarbazide are reported herein. These derivatives were evaluated against infection by the

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