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A novel GC-MS for the determination of Clopidogrel bisulfate in bulk and pharmaceutical dosage forms

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ABSTRACT

A simple, sensitive, precise and stability-indicating GC-MS method for the analysis of Clopidogrel bisulfate was developed and validated. The chromatographic separation was performed on Shimadzu -GCMS-QP2010 Plus device equipped with META-5X column (30.0 m X 0.32 mm X 0.25 mm), carrier gas was He, and gas flow rate 1.27 ml/min. Mass spectra were obtained by electron impact (EI) ionization at 70 eV, the qualitative analysis was made in Scan mode while the quantitative analysis was in SIM mode. The retention time of Clopidogrel bisulfate was about 17.2 min. Clopidogrel bisulfate was subjected to acid and alkali hydrolysis, oxidation and photo-degradation. The method was validated for different parameters. The results indicate that the drug was susceptible to degradation. All the peaks of degraded products were resolved from the active pharmaceutical ingredient with significant different retention times. As the method could effectively separate the drug from its degradation products, it could be employed as a stability- indicating method.

Key words: GC-MS; Clopidogrel bisulfate; assay validation; degradation; stability indicating.

INTRODUCTION

Clopidogrel bisulfate is a widely used antiplatelet drug worldwide. It is chemically known as methyl(s)-2-chloro phenyl (4,5,6,7-tetrahydro thieol [3,2-c] pyridine-5yl) acetate bisulphate (Fig. 1). Clopidogrel is an antiplatelet aggregation agent which selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor and blocks the subsequent ADP mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation (Jarvis and Simpson, 2000). It is used worldwide for chronic prevention of atherothrombotic events such as myocardial infarction, stroke, peripheral arterial disease, acute coronary syndrome, and cardio-vascular death (Bertand et al., 2000; CAPRIE Steering Committee, 1996; Yusuf et al., 2001).

Although the majority of the drug is hydrolyzed by esterase to an inactive carboxylic acid metabolite, the full anti-aggregating activity of the drug is achieved by biotransformation to 2-oxo Clopidogrel by cytochrome P450-1A. This intermediate metabolite is hydrolyzed, and it generates an active form which reacts as thiol reagent with the ADP receptor on platelets thus preventing the binding of ADP (Pereillo et al., 2002). The International Conference of Harmonization (ICH) guidelines require that analysis of stability test samples should be done by using stability indicating assay methods developed and validated after stress testing on the drug under a variety of conditions including hydrolysis across a wide range of pH values, oxidation, photolysis and thermal degradation. Therefore, there is a necessity to subject the drug to stress studies and establish stability indicating assay methods based on analysis of stressed samples (Shah et al., 2008). The United States Pharmacopoeia recommended a revers phase HPLC method with UV detection at 220 nm for determination of Clopidogrel bisulphate in tablets. Literature survey reveals that there are different types of assay methods for the determination of Clopidogrel bisulfate in pharmaceutical dosage forms. These analytical methods include chemometric (Rajput et al., 2008), spectrophotometric (Chaudhari et al., 2010), TLC (Antic et al., 2007), HPLC (Rashmin et al., 2008), HPTLC (Himani et al., 2003), LC-MS (Mitakos and Panderi, 2002), gas chromatographic (Kample and Venkatachalan, 2005) and voltametric methods (Dermis and Aydogan, 2010). The aim of the presented work is to develop simple, accurate, and stability-indicating method for the determination of Clopidogrel bisulfate in the presence of its degradation products after performing stress studies under a variety of ICH recommended test conditions. The presented study only deals with method development and validation using GC-MS technique.

MATERIALS AND METHODS

Drugs and chemicals: Working standard of Clopidogrel bisulfate (Purity 99.81%) was provided as a gift from Al-Razi Laboratories, Syria and used without further purification. All the other reagents used were of GC grade: Methanol (Sharlau, Spain), n-hexane, Hydrochloric acid (Surechem, England), Sodium hydroxide, Hydrogen peroxide, Filters 0.45 µm. Rotary evaporator (BÜCHI).The GC was Shimadzu -GCMS-QP2010 Plus device equipped with META-5X column (30.0 m X 0.32 mm X 0.25 mm), carrier gas was He, and gas flow rate 1.27 ml/min. Mass spectra were obtained by electron impact (EI) ionization at 70 eV.

Standard Preparation: Accurately weighed 100 mg of drug was dissolved in 100 ml of n-hexane. The resultant solutions were appropriately diluted to obtain final concentration in the range 5-25 µg/ml and chromatograms were run.

Preparation of Sample Solution: The sample solution of Clopidogrel bisulfate was prepared by taking 20 tablets of Clopidogrel bisulfate (Commercial dosage forms from the national market), and their average weight was calculated, The tablets were crushed to a fine powder, drug equivalent to 75 mg powder was transferred to a 100 ml volumetric flask and dissolved in methanol till the mark, the solution was sonicated for 10 min. with intermittent shaking then centrifuged at 10,000 rpm for 10 min. The centrifuged solution was filtered through 0.45µ filter. The filtered solution was appropriately diluted to obtain final concentration in the range 5 – 25 µg/ml and chromatograms were run.

Stress degradation of Clopidogrel bisulphate: Different kinds of stress conditions were employed on Clopidogrel bisulfate based on the guidance available from ICH Stability Guideline. The details of the stress conditions applied were as follow:

Preparation of acid and base induced degradation Product: Accurately weighed 100 mg of drug was dissolved in 100 ml of methanol. The drug was subjected to accelerated degradation under acidic and basic conditions by refluxing with 1N HCl and 1N NaOH, respectively, at 70°C for a period of 3 and 1 hr, respectively. The accelerated degradation in acidic and basic media was performed in the dark in order to exclude the possible degradation effect of light on the drug. The resultant solutions were evaporated by rotary evaporator, dissolved in n-hexane and chromatograms were run.

Preparation of hydrogen peroxide induced degradation product: Accurately weighed 100 mg of drug was dissolved in 100 ml of methanol. Subsequently, 10 ml of hydrogen peroxide 30.0% v/v was added and the solution was heated in boiling water bath for 1 hr till the removal of excess hydrogen peroxide. The resultant solutions were evaporated by rotary evaporator, dissolved in n-hexane and chromatograms were run.

Photochemical degradation product: Accurately weighed 100 mg of drug was dissolved in 100 ml of methanol. The photochemical stability of the drug was also studied by exposing the drug solution to direct sunlight for 12 h. The resultant solutions were evaporated by rotary evaporator, dissolved in n-hexane and chromatograms were run.

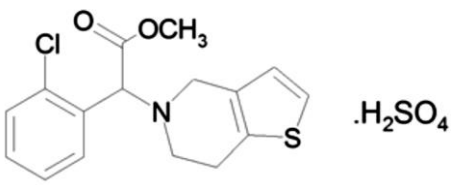

Figure.1.Chemical structure of Clopidogrel bisulfate

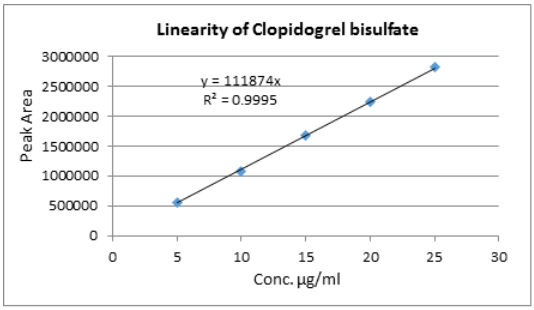

Figure.3.Linearity of Clopidogrel

RESULTS AND DISCUSSION

GC-MS analysis: The chromatographic conditions were optimized with a view to develop and validate the method for the determination of Clopidogrel bisulfate and to be a stability indicating method, as showed in Figure 2.

Analytical method validation: Method validation was performed under a variety of ICH and United States Pharmacopeia 30 recommended test conditions.

Linearity: Five standard solutions of Clopidogrel bisulfate were prepared with the concentrations (5, 10, 15, 20 and 25 µg/ml), each solution was injected six times in GC-MS. Figure (3) shows the linearity of Clopidogrel bisulfate with a correlation coefficient of 0.9995.

Range: Linearity, precision and accuracy were conformed in the interval (5, 10, 15, 20 and 25 µg/ml) for Clopidogrel bisulfate.

Accuracy: Concentrations of (5, 10, 15, 20 and 25 µg/ml) have been used to study the accuracy of Clopidogrel bisulfate **Table (1)**. Results indicate that the individual recovery of Clopidogrel bisulfate ranges from 96.872% to 101.450% with mean recovery of 99.661% and %RSD of 1.487%.

Precision:

Repeatability: The solution 15 µg/ml has been injected ten times. Standard deviation and relative standard deviation of the response (peak area) have been calculated and the results were illustrated in Table (2). The RSD% for repeatability of sample preparation is 0.64; this shows that the precision of the method is satisfactory as RSD% is not more than +2.0%.

Intermediate Precision: The RSD% for intermediate precision of the sample preparation is 1.026. This shows that the intermediate Precision of the method is satisfactory as RSD% is not more than +2.0%; Table (3).

LOD and LOQ: The calculated LOD (Limit of Detection) and LOQ (Limit of Quantification) were 5.13 µg/ml and 15.54 µg/ml respectively.

Stability indicating property: The chromatogram of no stress treatment sample (as control) indicated no additional peak **Figure (2).** A standard solution of Clopidogrel bisulfate was exposed to different kinds of degradation (acid, base, oxidation, sunlight) and injected in GC-MS. The chromatogram of acid degraded sample and alkali degraded sample presented one additional peak with retention time of about 15.35 min, but it's well resolved from the peak of Clopidogrel bisulfate with a significant difference in the retention time between the two peaks Figure (4). The chromatogram of H_2O_2 degraded sample illustrated two additional peaks at retention times 9.27 and 10.39 min and they are well resolved from the peak of Clopidogrel bisulfate Figure (5).There were no additional peaks in photo degraded sample of Clopidogrel bisulfate. As presented in all the chromatograms, the peak of Clopidogrel bisulfate is completely separated from the peaks of degradation products.

System Suitability: System suitability parameters were calculated and illustrated in Table (4).

Assay: The sample solution (Tablets 75 mg) was injected into GC-MS. The retention times was found to be 17.19 min for Clopidogrel bisulfate. The amount of drug present per tablet was calculated and the data are presented in Table (5).

Table.1.Accuracy of Clopidogrel

Conc. µg /ml	Area	Calculated Area	Calculated Conc. µg /ml	Recovery	AVR	RSD %
5	559837	5004174.339	5.004	100.083	99.736	1.915
5	567483	5072519.084	5.072	101.450		
5	546373	4883824.66	4.883	97.676		
10	1097265	9808042.977	9.808	98.080	97.439	0.623
10	1083748	9687219.551	9.687	96.872		
10	1089283	9736694.853	9.736	97.366		
15	1677875	14997899.42	14.997	99.985	100.019	0.521
15	1669984	14927364.71	14.927	99.515		
15	1687465	15083620.86	15.083	100.557		
20	2259847	20199930.28	20.199	100.999	100.209	0.709
20	2229047	19924620.56	19.924	99.623		
20	2237589	20000974.31	20.000	100.004		
25	2823746	25240413.32	25.240	100.961	100.903	0.461
25	2834251	25334313.6	25.334	101.337		
25	2808376	25103026.62	25.103	100.412		
Mean				99.661		1.487

Table.2.Repeatability of Clopidogrel

Conc. µg/ml	Area	AVR	STDV	RSD %
15	1677875			
15	1669984			
15	1687465			
15	1676452			
15	1656574	1676840	10739.95	0.64
15	1677364			
15	1668542			
15	1687465			
15	1689837			

Table.3.Intermediate precision of Clopidogrel

No. of Injection	Conc. Micro/ml of assay Preparation	Area
Solution A		
1	15	1677875
2	15	1669984
3	15	1687465
4	15	1676452
5	15	1656574
6	15	1677364
Solution B		
1	15	1708542
2	15	1687465
3	15	1658542
4	15	1687465
5	15	1709863
6	15	1716235
Average		1681599.18
SD		17260.034
RSD %		1.026

Table.4.System Suitability of Clopidogrel

Acceptance Criteria	Value	Result
RSD% of Area ≤ 1	0.741	Pass
RSD% of RT ≤ 1	0.543	Pass
Assymetry 0.8 - 1.5	0.95	Pass
Plate Effe. 50 % > 2000	18700.8	Pass

Table.5.Assay of Clopidogrel bisulfate in commercial formulation

Brands	Amount of Drug (mg/Tab) Labeled	Estimated*	Recovery %	RSD % n=3
A	75	75.47667	100.63	0.74
B	75	74.56	99.41	0.43
C	75	74.17	98.89	0.39
D	5	75.12	100.16	1.49

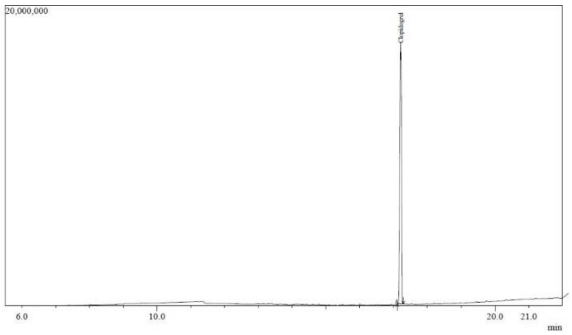

Figure.2.GC-MS chromatogram of Clopidogrel bisulfate

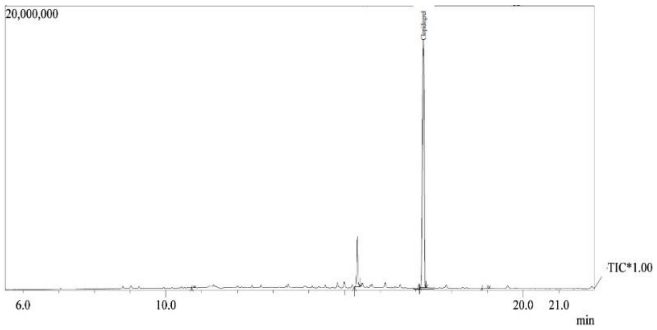

Figure.4.Acidic and basic degradation of Clopidogrel

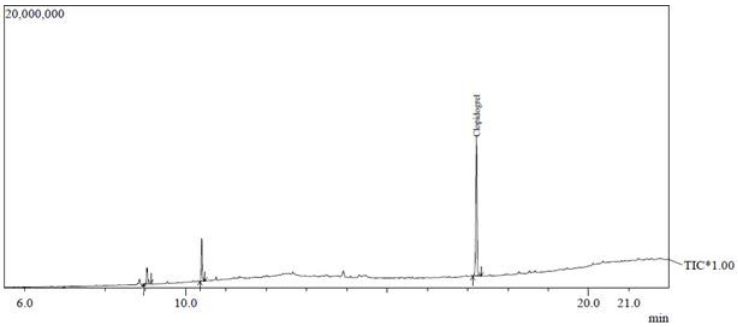

Figure.5.Degradation of Clopidogrel under 30% H_2O_2

CONCLUSIONS

The GC-MS described in this report appears simple, precise, accurate and stability-indicating for the analysis of Clopidogrel bisulfate in raw material and in pharmaceutical dosage forms. As the method separates the drug from its degradation products, under all stress conditions using HCl, NaOH, H_2O_2 and sunlight, it can be employed as a stability indicating one.

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