SYNTHESIS AND CHARACTERIZATION OF CLOPIDOGREL RELATED IMPURITY A

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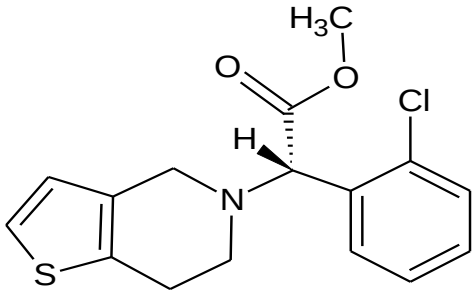
Abstract- **An antiplatelet medication called clopidogrel bisulfate is used to treat platelet aggregation and stop blood clots. Six potentially related chemicals that are generated at different phases of the synthesis of clopidogrel Bisulphate and have been identified throughout the laboratory process development of the drug. Clopidogrel related compound A is identified and synthesized. The current paper describes the effective synthetic techniques u to synthesize this related compound. The pathways for the synthesis of these chemicals and analytical characterization is also described in the current work.**

Keywords: **Clopidogrel, HPLC, IR, Mass Spectroscopy**

INTRODUCTION:

Clopidogrel is an oral **antiplatelet agent** used in treatment of **coronary artery disease, peripheral vascular disease, and cerebrovascular disease.**

- **Structure**



- **IUPAC Name**: methyl (2-chlorophenyl)(6,7-dihydrothieno[3,2-*c]pyridin- 5(4H)-yl)acetate.*
- **Molecular Formula**: $C_{16}H_{16}ClNO_2S$

Clopidogrel, an antiplatelet agent structurally and pharmacologically similar to ticlopidine, is used to reduce atherosclerotic events such as myocardial infarction, stroke, and vascular death in patients who have had a recent stroke, recent MI, or have established peripheral vascular disease.

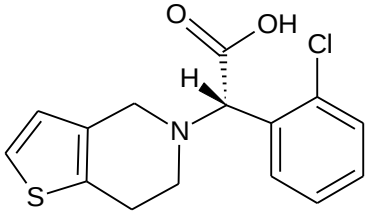
2.4.3 Mechanism of Action

The active metabolite of clopidogrel prevents binding of adenosine diphosphate (ADP) to its platelet receptor, impairing the ADP-mediated activation of the glycoprotein GPIIb/IIIa complex. It is proposed that the inhibition involves a defect in the mobilization from the storage sites of the platelet granules to the outer membrane. No direct interference occurs with the GPIIb/IIIa receptor. As the glycoprotein GPIIb/IIIa complex is the major receptor for fibrinogen, its impaired activation prevents fibrinogen binding to platelets and inhibits platelet aggregation. By blocking the amplification of platelet activation by released ADP, platelet aggregation induced by agonists other than ADP is also inhibited by the active metabolite of clopidogrel.

Related Impurities

Related Compound A

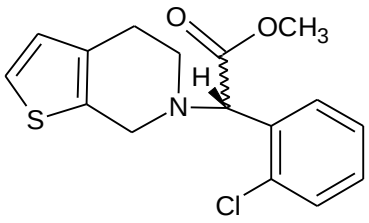
(+)-(S)-(o-chlorophenyle)-6,7-dihydrothieno(3,2-c)pyridine-5(4H)-aceticacid hydrochloride.



Relative retention time – 0.5

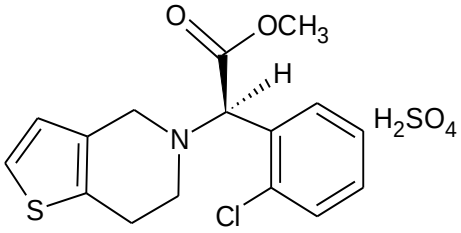
Related Compound B

(±)-(RS)-Methyle[(o-chlorophenyle)-4,5-dihydrothieno(2,3-c)pyridine-6(7H)]acetate,hydrogen sulphate .



Relative retention time of E_1 :0.8
Relative retention time of E_2 :1.2

2.5.3 Related Compound C
(-)-(R)-methyle-(o-chlorophenyle)-6,7-dihydrothieno(3,2-c)pyridine-5(4H)- acetate,hydrogen sulphate.



Clopidogrel Related Compound A: Not more than 0.200 % w/w
Clopidogrel Related Compound B: Not more than 0.300 % w/w
Clopidogrel Related Compound C: Not more than 1.000 % w/w
Any other impurity: Not more than 1.500 % w/w
Total impurity: : Not more than 1.500 % w/w

MATERIALS AND METHODS :
Synthesis of Clopidogrel Related Compound A
1.Prepration of (R*S*) -(±)-(2-chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridin-5-(4*H*) acetic acid) (racemic Clopidogrel acid) by Clopidogrel bisulphate.

PART-I-Prepration of Clopidogrel free base
268 g (0.63 mole) Clopidogrel bisulphate , 1340 ml (5 vol.) MDC were taken in a 3L 4NRBF and stirred for 15 minutes. Chilled it to 0-10°C. pH was Adjusted to alkaline (8-9 pH) using saturated Sodium bicarbonate solution. MDC layer was separated and washed with 200 ml water. The MDC layer was dried on anhydrous sodium sulphate and concentrated under vacuum on rotavapour at 50-55°C to get yellow color viscous oil.
PART-II- Preparation of racemic Clopidogrel acid
The oil obtained in above experiment(free base of Clopidogrel bisulphate) ,80 g (2 mole) NaOH,3000 ml methanol and 80 ml water were charged into a clean and dry 5 liter 4 NRBF. The reaction mixture was refluxed at about 60-66°C for 24 hours. TLC was checked for absence of starting material i.e Clopidogrel free base.
After completion of the reaction methanol from reaction was distilled out completely under vacuum at below 50-55°C.After distillation of methanol a residue is obtained which is sodium salt of acid, 1000 ml purified water was charged to dissolve the salt. The pH of the reaction mixture was adjusted 5-6 by using 35% aqueous hydrochloric acid. Extracted the acid using 3×3 L MDC.The 70% of MDC layer was distilled out 70% under vacuum at temperature 40-45 %. A solid precipitate was observed. The reaction mixture was stirred for 15 minutes at 25-35 to get complete precipitation of solid.
Further the reaction mixture was chilled to 0-5◦C followed by stirring for 15 minutes. The solid was filtered and solid cake was washed with 2×125 ml chilled MDC. The solid was suck dried and kept into oven for 2 hours at 50-55°C for drying.
Yield (Dry wt.)= 112gm.,% Yield =51.07%,M.P- 199.5-201.5°C
SOR at $[\alpha]_D^{20}$ in 1% methanol=0.0972
2. Preparation of (+)-(S)-amine salt of (o-chlorophenyle)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid by Resolution of racemic Clopidogrel acid by S(-) phenyl ethyl amine.
2576 ml (23 vol.) acetonitrile , 112 g (0.3253 mole) racemic Clopidogrel acid were charged into a clean and dry 3L 4NRBF followed by stirring for 10 minutes at 25-30°C. 98.55 g (0.8132 mole) S(-) phenyl ethyl amine was charged to the above reaction solution at 25°C followed by stirring for 6 hours . A solid precipitation was observed. Further reaction mixture was stirred for 18 hours for complete precipitation of solid .The reaction mixture was chilled to 0-5 °C. The solid was filtered and washed with 100ml chilled acetonitrile.The wet solid was subjected to drying for about 6 hours at temperature 55°C .
Yield = 69g, %Yield=49.44%, M.P.-145.2-146.6°C
SOR at $[\alpha]_D^{20}$ in 1% methanol=16.8829
3. Purification of (+)-(S)-amine salt of (o-chlorophenyle)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid by use of acetonitrile.
61.9 gm. (0.14428 mole)Amine salt of Clopidogrel acid and 2576 ml (23 vol.) acetonirile were charged in to 3L 4NRBF followed by stirring for 15 minutes .The reaction mixture was refluxed at 80-85°C up to get clear solution . After getting clear solution the

reaction mixture was filtered through filter paper in hot condition to remove inorganic impurities because these impurities cause deviation in specific optical rotation .

This filtrate was transferred in to another flask and cooled it to room temperature followed by cooling up to 0-5°C.The solid was separated out and this solid was filtered. Suck dried the solid and subjected for drying at 50-55°C in hot air oven.

Yield= 43 gm,% Yield=51.07%,M.P-145.2-146.2°C

SOR at $[\alpha]_D^{20}$ in 1% methanol=32.8231

3. **Preparation of (+)-(S)-(o-chlorophenyle)-6,7-dihydrothieno(3,2-c)pyridine-5(4H)-acetic acid hydrochloride.**

The 43 g amine salt of racemic Clopidogrel acid and 500 ml ethyl acetate was transferred into a dry and clean reactor and dry hydrochloride gas was passed in to reaction mass at 0-5°C in ethyl acetate solvent. Solid was separated out. The reaction mass was filtered and the solid was subjected for drying for 6 hours at 50-55°C

Yield: 30 gm.,%Yield=86.95%,M.P.-134.8-136.8°C

SOR at $[\alpha]_D^{20}$ in 1% methanol=38.8231

4. Purefication of (+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid hydrochloride.

30 gm. Of S(+) Clopidogrel acid was dissolved in 210 ml acetone and heat it up to 60°C then 90 ml water (70:30) was added into it to get a clear solution. After getting the clear solution it was chilled to room temperature followed by cooling at 0-5 °C .the solid got precipitated and filtered through filter paper. The solid was washed with 2×50 ml chilled acetone. The solid was suck dried and subjected for drying at 50-55°C.

Yield=27gm,% Yield=90.0%

SOR at $[\alpha]_D^{20}$ in 1% methanol

RESULTS AND DISCUSSION

Analytical Evidence of Synthesis of Clopidogrel Related Compound A

High Presser Liquid Chromatography Analysis

Instrument : Waters alliance 14

Column: Inertsil ODS 250×4.6 mm,5 µm

Mobile Phase: Gradient, Acetonitrile (Solvent A) and buffer (Solvent B) (0.02 M KH_2PO_4 in 1000 ml water)

Injection Volume: 10 µl

Run time: 40 minutes

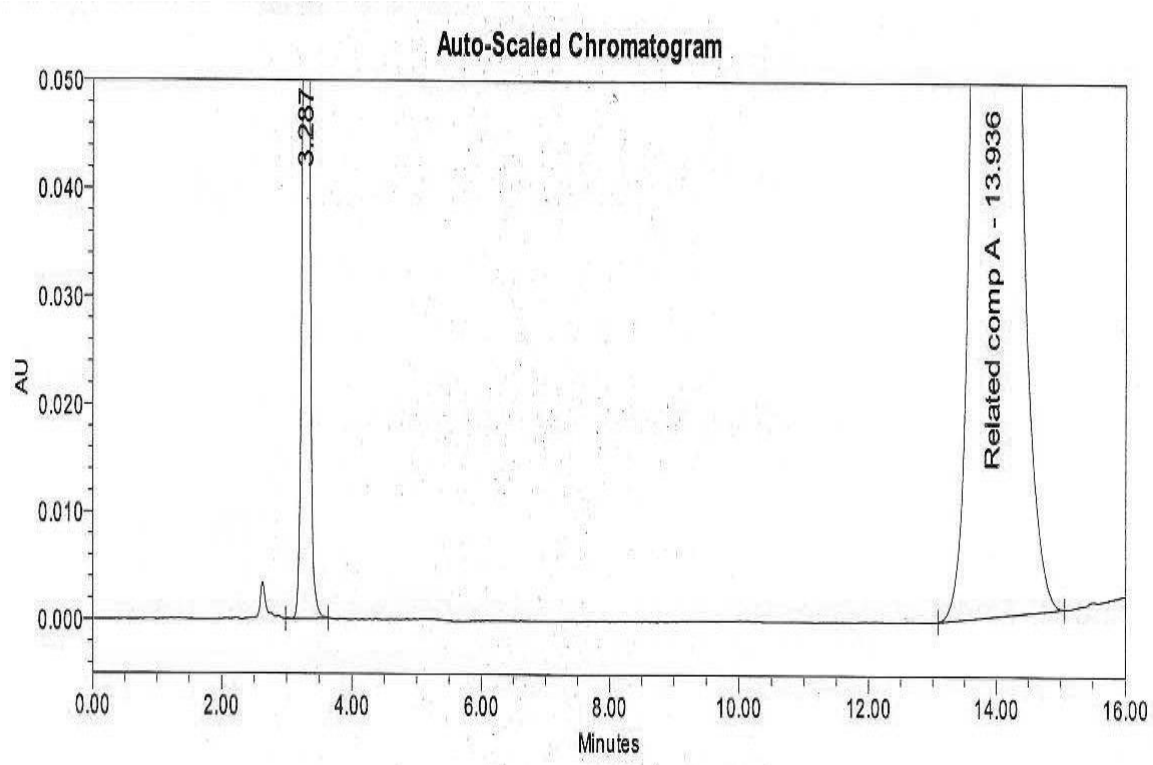
Flow rate: 1 ml/min

Detector: UV detector at 220 nm

Gradient table:

S.NO.	Time	Flow	%A	%B
1	0.0	1.00	20.0	80
2	10.0	1.00	20.0	80
3	20.0	1.00	80.0	20
4	30.0	1.00	80.0	20
5	31.0	1.00	20.0	80
6	40.0	1.00	20.0	80

HPLC Spectra of Clopidogrel Related Compound A



Peak Results

	Name	RT	Area	% Area
1		3.287	803597	3.172
2	Related comp A	13.936	24534262	96.828

Figure.1: HPLC spectra of USP related compound A

Remark: Clopidogrel related impurity shows the retention time at 13.936.It has 96.8% HPLC purity.

Overlapped HPLC Spectra for Clopidogrel Related Compound and USP Reference Standard

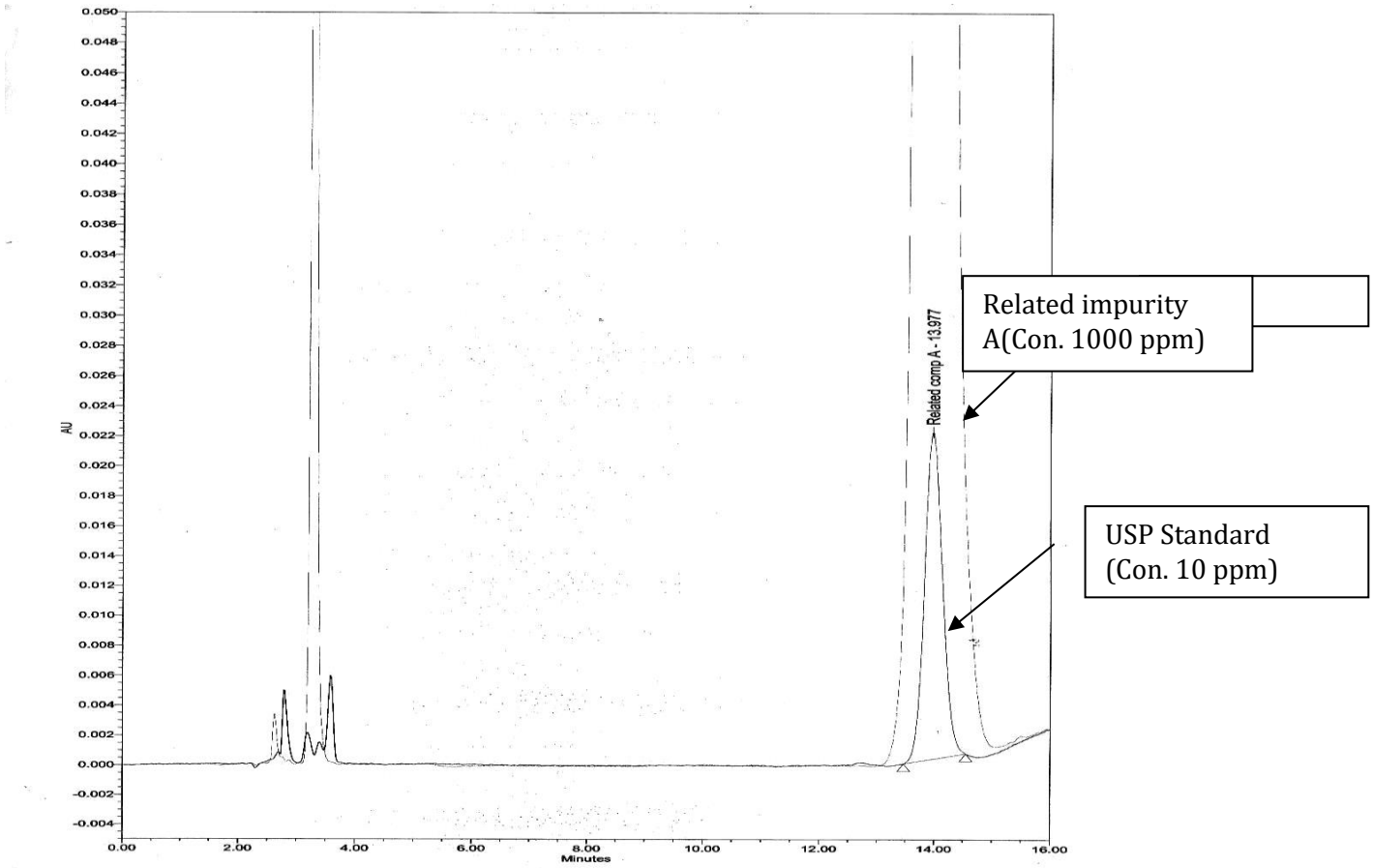


Figure.2: Overlapped HPLC spectra of Clopidogrel related compound A and USP Clopidogrel related compound A (Reference standard)

Remark:This spectra shows the same retention time of Clopidogrel related compound A as per standard and it confirms the synthesis of clopidogrel related compound A.

MASS SPECTROSCOPY :

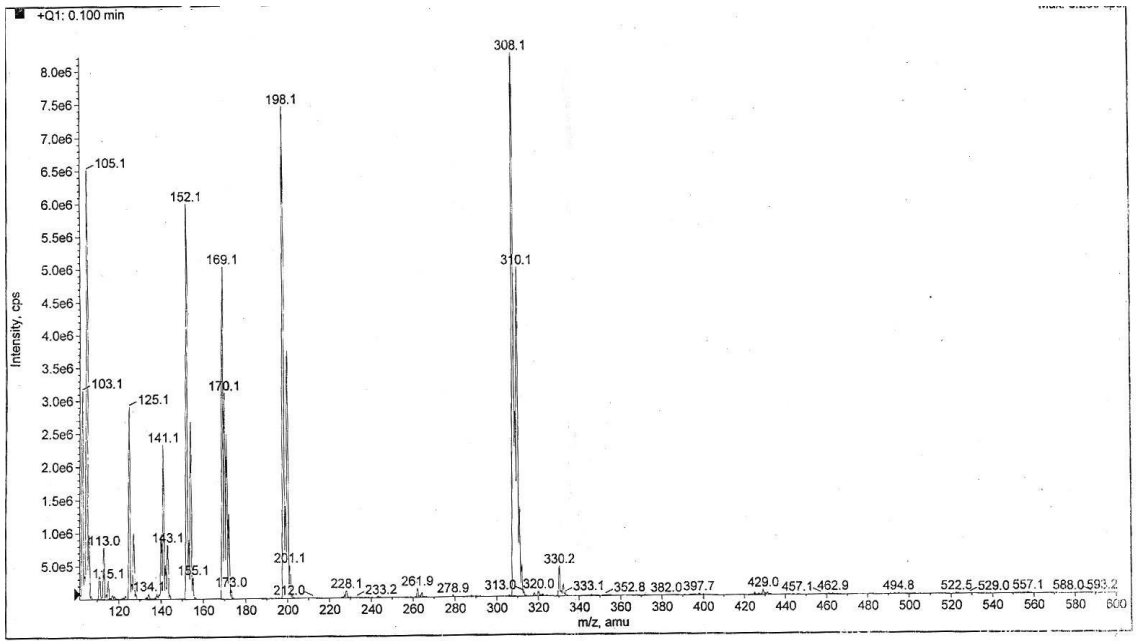


Figure.3: Mass Spectra of Clopidogrel related compound A

Observation: The expected mass is 344.75
The observed mass is 308.1 which is (M+1)
The actual mass is 307.1

The mass of HCl (36.45) is not shown in mass data.
(Considering the mass of HCl the actual mass of Clopidogrel acid hydrochloride is 343.75 amu.)

Due to presence of isotope of chlorine it shows the additional peak of 310.1 also.
Remark: Mass confirmed.

NMR SPECTRA :

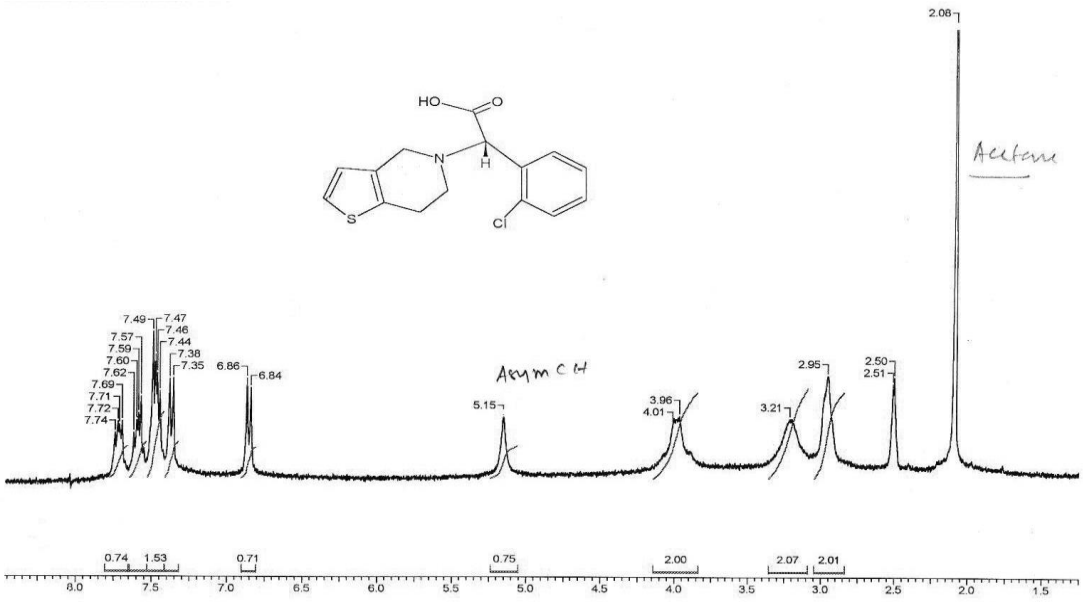


Figure..4: NMR Spectrum of Clopidogrel related compound A

The NMR spectrum of Clopidogrel related compound A is as follows:

Sr. No.	splitting	Chemical shift(δ) in ppm	No. of Protons	Functional groups
1	Multiplet	6-8	6H	Aromatic protons
2	**Singlet**	**5.15**	**1H**	**Asymmetric proton**
3	Triplate	3-4.5	6H	CH_2

Observation: This NMR Spectra shows the asymmetric protone at δ 5.15 which is the characteristic peak in the S(+) Clopidogrel acid and differs it from racemic Clopidogrel acid. Major signals are complying with the structure . Signal for COOH could not find at down field due to presence of the acetone solvent.
Remark: Proton NMR (^{1}HNMR) Spectrum of S(+) Clopidogrel acid hydrochloride complies with the structure.

IR Spectra of Clopidogrel related compound A:

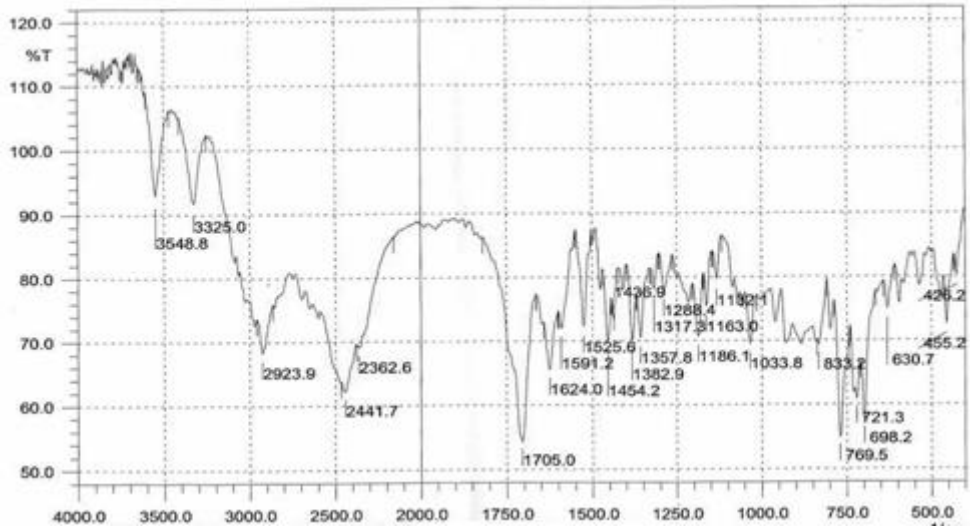


Figure..5:IR Spectrum of Clopidogrel related compound A.

The IR spectrum of Clopidogrel related compound A is as follows:

Sr. no	Bond	Mode	Relative strength	Wave number(cm^{-1})
1	C-H	Stretching	S	2700-3300
2	C-H	Bending	M	1375
3	Aromatic C-H	Stretching	M	3080
4	C-C	Stretching	M-S	2960-2850
6	C=O	Stretching	S	1700-1680
7	O-H	Stretching	S	3600-3200
8	C-O	Stretching	S	1250
9	C-Cl	Stretching	S	800-600
10	O-H	Bending	S	1400 &920

Remark: IR Spectra confirms the structure of the molecule.

CONCLUSION: We have developed improved process for Clopidogrel Related Compound A , which found to be more compatible with industrial scale and has significant advantages over the existing synthesis.

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