Synthesis And Antimicrobial Study of Fe (II) Complex of Schiff Base Derived From 4- Acyl Antipyrine and Substituted Aniline

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ABSTRACT

The incidence of drug- resistant microbial infections is a growing concern worldwide, necessitating the development of novel antimicrobial agents that would break barrier of resistance, guarantee safety and potency of pharmaceutical products. The chemical synthesis and antimicrobial studies of Iron (II) complexes of Schiff base derived from acetyl chloride antipyrine (4-acyl antipyrine) were carried out using substituted aniline (2-hydroxylaniline) was one such attempts of development of new molecular compounds capable of breaking barrier of resistance. The primary ligand, Schiff base ligand and their metal complex was characterized using spectroscopic techniques ranging from IR, UV-Vis, GCMS. The melting point, molar conductivity, elemental composition was determined. Interestingly, all the synthesized compounds were obtained in good yield (76%-86%). The molecular ion peaks (M^+) indicating the molecular weight of the synthesized ligand and metal complex were detected using the various fragments produced by the ligands and metal complexes based on their mass to charge ratio obtained from GC-MS Spectra. Octahedral geometry was observed for Fe (II) complex. The IR absorption showed characteristic behaviour in the sense that the v(C=N) found in SLOA and the metal complexes with frequency range of (1,582.90cm^{-1}) indicate that imine group/Schiff base is formed. The absorption band assigned to C=O in the ligand and metal complexes are as follow 1,700.65cm^{-1} and 1,623.00cm^{-1} respectively. The Schiff base ligands SLOA (I,700.65cm^{-1}) showed a notable shift to higher wavenumbers indicating increase C=O bond strength due to coordination with metal through its oxygen or electron withdrawal in the Schiff base framework. Metal complexes show C=O stretching shifted back to lower frequency value (1.633.54cm^{-1}) suggesting co-ordination of oxygen to metal. The metal complexes demonstrated great antimicrobial efficiency on test organisms of both bacteria (Salmonella typhi, Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes) and fungi (Candida albicans) more than the ligand due to lipophilicity of the chelated complexes which retarded their growth process. This study showed that synthesis and complexation have taken place and the knowledge gained will help to advance the course of bioinorganic and inorganic chemistry as well as incorporating ligands and metal complexes into antibiotic drugs production.

Keywords: Schiff base, Antimicrobial, Metal complex, 4-acyl antipyrine, Fe(II)Complex, Coordination.

INTRODUCTION

The coordination chemistry of metal complexes plays a vital role in biological system of organisms. Transition metal complexes are important in catalysis, material synthesis, photochemistry and biological systems. The synthesis of ternary complexes mainly involves the interaction of metal ion with two or more different ligands. Recently there has been considerable interest in mixed chelation because it occurs commonly in biological fluids, which contain millions of potential ligands which are likely to compete in vivo for metal ions. It is well known that the ternary coordination complexes play an important role in biological processes as exemplified by many instances in which enzymes are known to be activated by metal ions [1]. Ternary complexes have also been implicated in the storage and transport of active substances through membranes [2] and these phenomena are strongly dependent on the formation of these species and the electronic configuration of metal ion concerned. The stability constant and complexation behaviour of Fe (II) complexes with various ligand have been studied extensively [3].

The coordination number is the number of ligand-binding sites on the metal ion. The bond between the metal ion and the ligand, where the ligand supplies both electrons, is known as a co-ordinate covalent bond. A co-ordinate bond, also known as a dative covalent bond is a covalent bond, a shared pair of electrons in which both electrons to be shared originate from the same atom. Transition metals are up to all sorts of unusual activities in the chemical world. Like giving colour to compounds or performing vital functions in living things. Many of their unique abilities have to do with their electron configurations. Because they are so special, we often find transition metals as the center of attention, literally. In coordinated compounds the transition metal is in the middle of the complex ion [4].

Transition metals involved in the complex ion have two sets of valence electrons participating in bonding. The first set of bonding electrons is called primary valence, and it is the oxidation number of the metal. The oxidation number can be determined by looking at the charge on the transition metal ion. Iron (Fe), for example, has an oxidation number of 2. Sometimes this number must be inferred based on the overall charge of the complex ion. The primary valence electrons are involved in typical ionic bonds [5]. The second set of transition metal valence electrons are called secondary valence, usually referred to as the coordination number. The secondary valence electrons are involved with bonding with the ligands. The coordination number indicates the number of ligands that a metal ion is bonded to [6]. Ligands bond to transition metals by sharing a lone pair of electrons. This type of interaction is a Lewis acid-base reaction, where the metal ion is the Lewis acid and the ligand is the Lewis base. The resulting bond in which one species donates both bonding electrons is called a coordinate covalent bond [7].

Pyrazolone derivatives are also used in preparing dyes and pigments [8]. 2,3-dimethyl-1-phenyl-5-pyrazolone (antipyrine) has been discovered as antipyretics of the quinoline type [9]. This discovery initiated the beginning of the German Drug Industry that dominated the field for approximately 40 years.

5- pyrazolone as a widely used precursor to variety of compounds, documented well for their numerous applications such as products and intermediates in analytical, agricultural, biological and pharmaceutical chemistry [10,11,12]. Some of them also serve as important pharmaceutical agents including antipyrine and its congeners. With continuous evaluation for their pharmacological properties like analgesic [13], potential antipyretic, anti-nociceptive and antioxidant activities [14]. Recently, acylpyrazolone have been reported to have a multidrug resistance modulating activity [11]. Benzoyl pyrazolone particularly, is potential antiprion agents [15]. An antiprion agent is a compound or drug designed to target and combat prions, which are abnormally folded proteins responsible for causing fatal neurodegenerative diseases known as prion diseases [16]. These antiprion agents work by inhibiting the misfolding of normal cellular prion protein into the infectious, pathogenic form [17]. The presence of fragment azomethine group (-N=CH-R) in Schiff bases is known for its biological activity [18]. Many reports exist on structure- activity relationship of the class of this compound, therefore it becomes worthwhile to continue to further investigate in this molecule.

Iron(II) complex, in particular, have become of research interest because several Fe(II)– 4 acyl antipyrine complex has demonstrated promising antibacterial and antifungal result in vitro, and in some cases, improved activity against drug-resistant strains.[9] These findings justify exploring Fe(II) chelates of new Schiff base ligand as potential antimicrobial agents. This can be attributed to the concept called lipophilicity which is the ability of the metal complex to penetrate or dissolve the cell membrane of microorganism hence retards the growth of the microorganism [18].

This research work focuses on Synthesize and characterize Fe (II) complexes of Schiff base ligand derived from 4- acyl antipyrine using substituted anilines (2-hydroxylaniline) and the determination of the antimicrobial activities of the synthesized ligand and metal complex.

Experimental

The Apparatus and Reagents

The reagents used in this work are analytical grade and they are as follows; Chloroacetyl Chloride (Sigma – Aldrich), Acetyl Chloride (Sigma-Aldrich), Antipyrine (Sigma -Aldrich),2- amino aniline (Sigma-Aldrich), 2-Hydroxyl aniline (Sigma- Aldrich), Cobalt (II) Acetate (J.T. Baker), Nickel (II) Acetate (J.T.Baker), Iron (II) Acetate (J.T. Baker) and Dioxane (Sigma- Aldrich), HCl(Sigma- Aldrich), Calcium Hydroxide (Sigma-Aldrich), n-Hexane (Sigma- Aldrich),Carbon tetra chloride(Sigma- Aldrich), Deionized water. The solvents were ethanol (J.T. Baker), Methanol (J. T. Baker), Acetone (J. T. baker) and Ether (J. T. Baker)

The electronic equipment: Fourier Transform Infrared (FTIR) (Nicolet Is5, Thermo Fisher Scientific USA), Electronic weighing balance (Ohaus,Adeventurer),Beakers(Pyrex),Conical flasks(Pyrex),Bunsen burner (Fisherbrand),Waterbath (Grant), Filter paper (Fisherbrand),Stuart MP 3, Agilent 7977 Gas Chromatograph,5973D Inert Mass Spectrometer (Thermo Scientific USA),Conductivity meter (HACH HQ40D), Elemental Analyzer CE -440 (Exeter Analytical Inc.UK).

The bacteria Species: Salmonella typhi (Gram negative bacteria) Escherichia coli (Gram negative bacteria), Staphylococcus aureus (Gram positive bacteria). Streptococcus pyogenes (Gram positive bacteria), Candida albicans (Fungi, yeast) were obtained from the Reference Laboratory Section of Gomecs-everglad Laboratories, Owerri, Imo State, Nigeria. The organisms were maintained on Nutrient Broth for 24 hours.

Synthesis of 4- acyl antipyrine:

9g (0.05mol) of antipyrine (2,3-dimethyl-1-phenylpyrazolone-5) was dissolved in hot dioxane (70cm^3) placed in a round bottom flask equipped with a stirrer, separating funnel and reflux condenser. Calcium hydroxide (7.00g,0.1mol) was added to this solution, followed by acetyl chloride (5ml, 0.07mol) added drop wise. The reaction mixture became thick paste and was refluxed for 2 hrs. and allowed to cool. The mixture was poured into hydrochloric acid (200cm^3). The cream-coloured crystals obtained were filtered and then recrystallized from cold ethanol- water acidified with HCl to destroy any undecomposed calcium complex and recrystallized. The yield was 80%, melting point 116^{0}C and was labelled LOA.

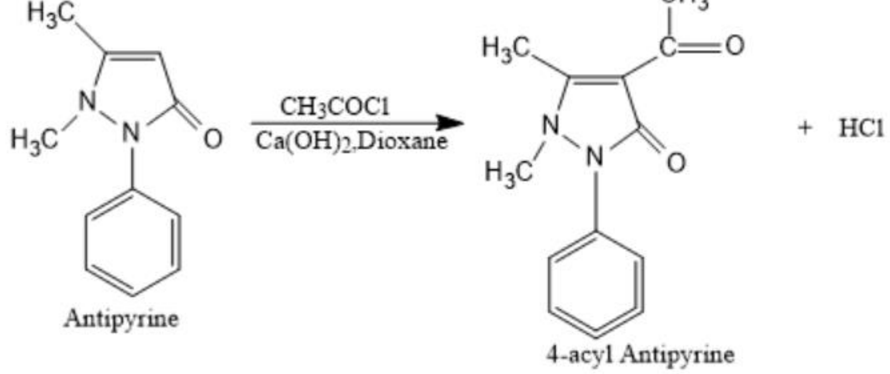


Fig 2.1: Scheme of Reaction for the Synthesis of 4-Acyl Antipyrine.

KEY:

LOA = Ligand of acetyl chloride antipyrine

LOC = Ligand of monochloroacetyl chloride antipyrine

Synthesis of Schiff base ligand from 4-acyl antipyrine and 2- hydroxyl aniline:

2.18g, 0.02mol of 2-hydroxylaniline was dissolved with 150ml absolute ethanol in 500ml round bottomed flask, to this solution was added dropwise 2.68g, 0.03mol of LOA in 30cm^3 of absolute ethanol over 30minutes while stirring. Stirring continued for another 30minutes and mixture refluxed for 3hrs.The resulting solution was allowed to cool and filtered to remove the solvent and the solid residue was washed with cold ethanol and then recrystallized with a mixed solvent of methanol, ethanol and acetone in the ratio of 1:1:1. The yield was 86% and melting point 232^{0}C. The Schiff base formed was labelled SLOA.

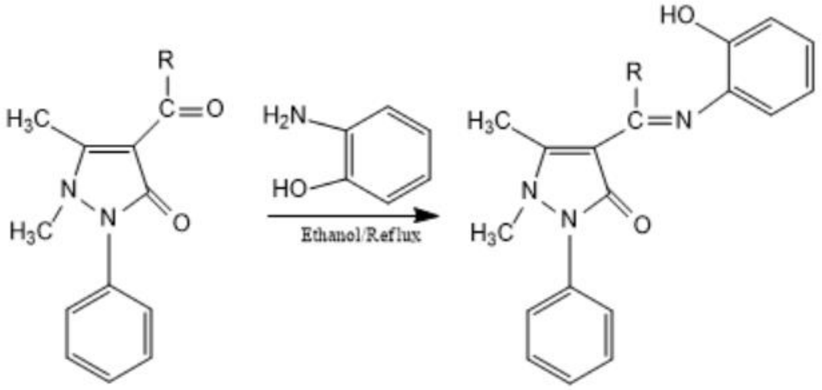


Fig 2.2 Scheme of Reaction for the Synthesis of the Schiff Base

KEY:

LOA = Ligand of acetyl chloride antipyrine

LOC = Ligand of monochloroacetyl chloride antipyrine

SLOA = Schiff base ligand of 2- hydroxylanilne

SLOC = Schiff base ligand of 2- aminoaniline

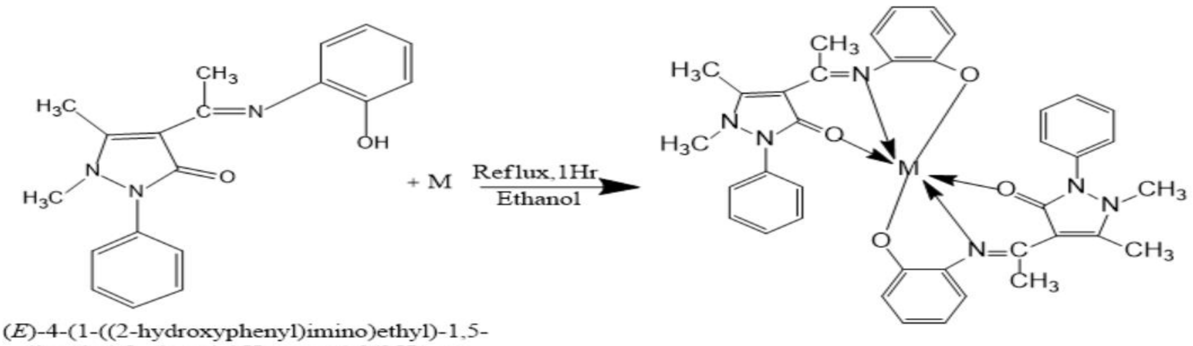
Synthesis of metal complexes:(M= Fe(II))

1 mmole solution of the metal acetate, M(II) $(OAC)_2$: 0.216g Fe (II) $(OAC)_2$ was placed in boiling ethanol solvent. I mmol solution of the Schiff base Ligand (SLOA 0.419g) was added then, a few drops of piperidine were also added as a precursor. The whole mixture was refluxed for 1 hour. It was then allowed to cool to deposit-coloured metal complexes. The precipitate was washed with the cold methanol and cold ether, allowed to dry in oven at a temperature of 50^{0}C. Metal complexes formed was labelled SLOAFe. The yield and melting point were SLOAFe 76%, 280-284^{0}C.

Instrumental analysis:

The molar conductivity measurement of the samples was carried out with a 10^{-3}m solution of the sample in absolute ethanol, at 25±0.5^{0}C. The conductivity was determined using conductivity meter (HACH HQ40d). The FT-IR Spectra of the samples were obtained in the 400-4000 cm^{-1} range using Fourier Transform Infrared (FTIR) (Nicolet Is5, Thermo Fisher Scientific USA), equipped with KBr optics and complimentary ATR diamond accessories. The acquired interferogram was converted into a spectrum by Fourier Transformation. In other to achieve good balance ripple size and resolution, the Happ Genzel function was used for Apodization (used in Hannwindow in fast FT analyzer to smooth the discontinuities at the beginning and end of the sampled time record). Agilent micro lab Expert FTIR Spectrometer software was used to acquire and process the data. The C, H, O and M(Metals) Contents of the samples were determined by flash combustion, using elemental analyzer (CE-440 Elemental Analyzer,Exeter Analytical Inc, UK). Sample weight used for the determination ranged from 1.0-1.5mg. The combustion and reduction were 975 and 600^0C respectively while the oven temperature was 81^0C. The chromatographic column Parapak PQS column, while the detector was thermal conductivity detector. The combustion was calculated from the second stage after pyrolysis and subsequent formation of Carbon monoxide (CO). The instruments used for the determination of GCMS was Agilent 7977 Gas Chromatograph, coupled to 5973D Inert Mass Spectrometer (with triple axis detector) with electron-impact source.

Complexation reaction of Schiff base with metals to form complexes (proposed structures)



(*E*)-4-(1-((2-hydroxyphenyl)imino)ethyl)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one

M(II) Complex of Schiff base of 2- Hydroxylaniline

Where M= Fe

M = Fe(II) Complex of Schiff base of 2- Hydroxylaniline (SLOAFe)

RESULT AND DISCUSSION

The ligands and metal complexes maintain their characteristic coloration as seen in Table 3.0. Interestingly, the ligands and metal complexes gave good yield (76-86%) indicating that the method of synthesis was viable. When compared with the work of [19,37,35,38] it was evidence that the yield of this work is good. The melting point of the synthesized ligand was high and that of the metal complexes was higher which later melted and decomposed. The increases in melting point are attributed to the increase in mass of the formed complexes and thus provide evidence for complexation. The elemental composition when compared with that of [19, 25, 27,29,39] is relatively good.

The solubility test results for the prepared ligands and their metal complexes are presented in Table 3.1. The Ligand and metal complex are insoluble in Diethyl ether and n-hexane. LOA showed slightly soluble in methanol, ethanol, carbon tetra chloride. The Schiff base ligand (SLOA) showed strongly soluble in methanol, ethanol and in acetone and water it showed slightly soluble. Furthermore, the metal complexes showed moderately soluble, strongly soluble and slightly soluble in methanol, ethanol, acetone, carbon tetra chloride and water respectively [36].

The Characteristic infrared frequencies of the ligand and metal complexes are listed in Tables 3.2, 3.3 and 3.4 hence presented in Appendix 1 to 3. The IR Spectral data shows the following important bands such as v(C=O), v(OH), v(C=N), $v(C\text{-}CH_3)$, $v(C_6H_6)$, v(C=C), v(C-H), v(M-O) and v(M-N). The absorption band assigned to C=O in the ligand and metal complexes ranges from 1.700.65cm^{-1} to 1,613.73cm^{-1}. The Schiff base ligands SLOA (I,700.65cm^{-1}) showed a notable shift to higher wavenumbers indicating increase C=O bond strength due to coordination with metal through its oxygen or electron withdrawal in the Schiff base framework [37]. Metal complexes show C=O stretching shifted back to lower frequency values (1.623.54cm^{-1}) suggesting co-ordination of oxygen to metal found in work of [6,22,26,29,33].

However, the Schiff base SLOA has a frequency value (3,500.06cm^{-1}) assigned to v(OH) in the aromatic ring [6,34.35]. The absorption band assigned to C=N Stretching in the Schiff base ligand as seen in SLOA – 1,582.90cm^{-1} confirming Schiff base formation. In complexes, C=N band shift downward (e.g., SLOAFe - 1,513.91cm^{-1}) as seen in table 3.4 indicate consistent coordination via azomethine nitrogen to the metal [28,32]. The frequencies ranging from 2,998.95cm^{-1} to 2, 510 cm^{-1} are assigned to v(C-CH_3) bonding for the ligand and the metal complex [34,35,36]. There is strong indication of the formation of aromatic C=C bond in the ligands and complexes with values ranging from 1,446.38cm^{-1} to 1,430.10cm^{-1}[37,33]. The variation in frequency values in the complex can be attributed to subtle π- electron redistribution upon coordination. The frequency values of the range 2,998.95cm^{-1} to 2,842.10cm^{-1} was assigned to stretching C-H bond. The absorption band assigned to aromatic ring (C_6H_6) vibration in the ligand (LOA) are 813.54cm^{-1}. The Schiff base SLOA (800.62cm^{-1}) show slight down shift possible as a result of ring substitution effect. In complexes, the frequency values range from (881.20cm^{-1}) [6,19,30].

Moreover, there are strong evidence of the formation of v(M-O) and v(M-N) bond in the metal complex with assigned values as follows 790.33cm^{-1} and 560.34cm^{-1} respectively, indicating coordination of both oxygen and nitrogen donor atoms to the metal [6,20.21,23,25,31].

The UV-Vis spectrum of the Schiff base SLOA and metal complex (SLOAFe) were characterized mainly by one absorption and thus appear to have virtually identical spectra, and absorb in the near visible region around λ_1=278nm for the Schiff base Ligand and metal complex λ_1= 420nm. The absorptions of the Schiff base are ascribed to n → π*, then the absorption of the metal complex can be ascribed to d→d* which are shown in Table 3.5 and presented in Appendix 7 to 8

The mass spectroscopy of the primary ligands, Schiff bases and metal complexes under study are shown in the Table 3.6 and presented in Appendix 4 to 6.

The mass spectrum of primary ligands (LOA) showed a molecular ion peak at 230.1m/z. The Schiff base ligand molecular ion peaks (SLOA) where found at m/z 321.0. The Schiff base ligand SLOA showed a characteristic peak of 107.0m/z and 214 m/z representing the aniline and acetyl chloride antipyrine fragment ion indicating the stability of these fragment ions in the Schiff base ligand SLOA. The base peak of SLOA is 216.1 m/z which is the most intense (tallest) peak in the mass spectrum, due to the ion with the greatest relative abundance [6,24,32,30].

The metal complexes SLOAFe showed a characteristic peak of 106.0 m/z, 214.1 m/z, 320.1 m/z representing the aniline, acetyl chloride antipyrine and the Schiff base ligand fragment ion respectively. The fragment ion with peak 640 m/z showed that the co-ordination of the Schiff base ligand (SLOA) with the metal ion is in the ratio of 2:1. The molecular ion peaks of the SLOAFe 696.0 m/z.

All the molecular ion peaks of the primary ligand, Schiff base ligand and metal complex agreed or equivalent to their calculated molecular masses.

The antibacterial activity of the test component was evaluated using the paper disc diffusion method against different species of bacteria; Salmonella typhi (Gram negative), Escherichia coli (Gram negative), Staphylococcus aureus (Gram positive), Streptococcus pyogenes (Gram positive) [6]. The antimicrobial activities were presented in the Table 3.8,3.9 and 3.10 as seen below. The test samples showed pronounced activities against the test bacteria. The streptococcus pyogenes showed resistance in LOA, SLOA. SLOAFe, SLOA and LOA inhibited the growth of bacteria when compared with others. The metal complex showed great inhibition against test microorganisms because of lipophilicity which is the ability of metal complex to dissolve in lipids or soluble in fat, since the cell membrane of microorganisms are made up of lipids [6]. The lipophilic metal complex can penetrate the cell wall of the test organism and inhibit their growth.

Antifungal activity was evaluated using the paper disc diffusion method against Candida albicans. Generally, SLOAFe, SLOA and LOA inhibited the growth of fugal.

The test samples showed pronounced MIC (Minimum Inhibition Concentration) activities against the test micro-organism as seen in table 3.8. SLOAFe, SLOA, LOA showed MIC at the range of 500mg/ml to 125mg/ml against the test microorganism. Moreover, no MIC was recorded for LOA and SLOA against Streptococcus pyogenes and SLOAFe against Candida albicans.

Finally, the metal complex showed high inhibition against microorganisms when compared with the ligands.

Table 3.0 showing the physical characteristics of the synthesized compounds

S/N	Synthesized compounds	Yield (%)	Colour	Melting Point t(^{0}C)	Molecular Weight(m/z)	Molar Conductivity Ω^{-1} cm^{-2} mol^{-1}		Elemental Compositions (%)					
						In acetone	In ethanol	C	H	N	O	Cl	M
1	LOA $C_{13}H_{14}N_2O_2$	80	Cream yellow	116^{0}C	230.1	3.2	1.4	68.42 (67.23)	4.83 (4.46)	6.40 (6.30)	12.70 (12.20)	-	-
3	SLOA $C_{19}H_{19}N_3O_2$	86	Black	232^{0}C	321.0	2.6	1.5	58.42 (58.25)	5.74 (5.25)	7.86 (7.17)	12.88 (12.01)	-	-
5	SLOAFe $C_{38}H_{36}N_6O_4Fe$	76	Grayish Brown	280-284^{0}C	696.0	2.5	2.1	62.34 (61.02)	3.48 (3.22)	5.32 (4.89)	10.25 (10.36)	-	10.82 (10.52)

KEY

LOA= Ligand of acetyl chloride antipyrine

SLOA= Schiff base ligand of 2- hydroxylaniline

SLOAFe = Iron (II) Complex of Schiff base of 2-hydroxylaniline

( ) = Calculated

Table 3.1: Solubility Values of The Ligands and Metal Complex

	Methanol	Ethanol	Acetone	Diethyl Ether	Carbon Tetra Chloride	Water	n-Hexane
LOA	SIS	SIS	MS	Insol	SIS	SIS	Insol

SLOA	SS	SS	SIS	Insol	Insol	SIS	Insol
SLOAFe	MS	MS	SIS	Insol	SIS	SIS	Insol

KEY: SS: **Strongly Soluble;** MS: **Moderately Soluble; Insol: Insoluble;** SIS: **Slightly Soluble**

Table 3.2: Showing the FTIR Spectral data of LOA Ligand

Assignment of Bond	Frequency range in cm^{-1}	Functional Group
v(C=O) Stretch	1,613.73	Carbonyl group in Pyrazolone
v($C-CH_3$) Stretch	2,982.08	Alkyl methyl group in Pyrazolone
v(C_6H_6)	813.54	Aromatic ring vibration
v(C=C) Stretch	1,436.36	Aromatic
v(C-H) bending vibration	2,896.42	Aromatic ring

Table 3.3: Showing the FTIR Spectral data of SLOA Ligand

Assignment of Bond	Frequency range in (cm^{-1})	Functional Group
v(C=O) Stretch	1,700.65	Carbonyl group in Pyrazolone
v(O-H) Stretch	3,500.06	Hydroxyl group in Aromatic ring
v($C-CH_3$) Stretch	2,998.95	Alkyl methyl group in Pyrazolone
v(C_6H_6)	800.62	Aromatic ring vibration
v(C=N) Stretch	1,582.90	Imine group/Schiff base
v(C=C) Stretch	1,446.38	Aromatic C=C
v(C-H) Bending vibration	2,998.95	Aromatic ring

Table 3.4: Showing the FTIR Spectral data of the Metal Complexes (cm^{-1})

	v(C=O) Stretch	v($C-CH_3$) Stretch	v(C_6H_6)	v(C=N) Stretch	v(C=C) Stretch	v(C-H) Bending	v(M-O)	v(M-N)
SLOAFe	1,623.00	2,510.83	881.20	1,513.91	1,430.10	2,842.10	790.33	560.34

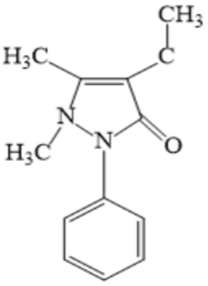
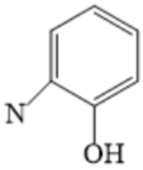
Table 3.5 UV-Visible Spectral for Schiff Base and Metal complex

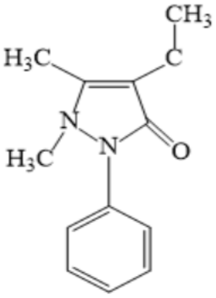
Compounds	**Wavelength(nm)**	**Elemental Transition**
SLOA	278	$n \rightarrow \pi^*$
SLOAFe	420	$d \rightarrow d^*$

Table 3.6: GCMS Analysis of Ligands and Metal Complex

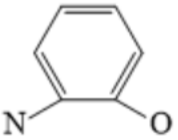
Compounds	Calculated Molecular Mass(g/mol)	Observed Molecular ion (M^+)	Base Peak(m/z)	Observed Fragment ions
LOA	230.1	230.1	148.1	54.2,84.1,120.1,185.1
SLOA	321.0	321.0	216.1	98.1,107.0,214.0,223.1
SLOAFe	695.9	696.0	640.1	106.1,212.0,214.1,320.0,640.1

Table 3.7: Summary of some Fragments in GCMS Spectrum

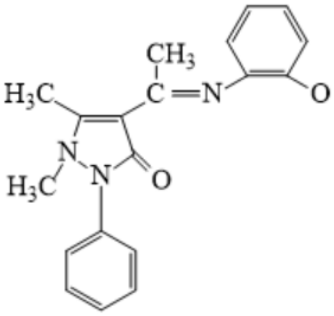
m/z	**Assignment/formula of compounds**	**Structure fragments**
321.0	**SLOA** $C_{19}H_{19}N_3O_2$	
214	$C_{13}H_{14}N_2O$	
107	C_6H_5NO	
696.0	**SLOA(M)** $C_{38}H_{36}N_6O_4(M_1)$	



214 $C_{13}H_{14}N_2O$



106 C_6H_4NO



302 $C_{19}H_{18}N_3O_2$

Where M = Fe, Ni, Co

M_1 = Fe

	Formulations/Zone of Inhibition(mm)				
Test Organisms	SLOAFe	SLOA	LOA	OFX	NY
Salmonella typhi	20	18	16	12	-
Escherichia coli	16	12	16	30	-
Staphylococcus aureus	20	18	28	30	-
Streptococcus pyogenes	14	10	8	26	-
Candida albicans	10	16	18	-	26

TABLE 3.8: ANTIMICROBIAL SUSCEPTIBILITY TESTING

Key:mm = Millimeter

SLOAFe, SLOA ETC = Sample codes

OFX = Ofloxacin

NY = Nystatin

Clinical Laboratory Standard Institute guideline for antimicrobial agents

- = Not determined

NI = No inhibition

R = Resistant (0 – 12 mm)

S = Susceptible (16 mm and above)

	Formulations/concentrations(mg/ml)		
Test organisms	SLOAFe	SLOA	LOA
Salmonella typhi	125	250	500
Escherichia coli	500	500	500
Staphylococcus aureus	125	250	125
Streptococcus pyogenes	500	ND	ND
Candida albicans	ND	500	250

TABLE 3.9: TEST FOR MINIMUM INHIBITORY CONCENTRATIONS

Key: mg/ml = Milligram per millilitre

SLOAFe, SLOA ETC = Sample codes

ND = Not detected

	Formulations/concentrations(mg/ml)		
Test organisms	SLOAFe	SLOA	LOA
Salmonella typhi	500	ND	ND
Escherichia coli	ND	ND	ND
Staphylococcus aureus	500	ND	500
Streptococcus pyogenes	ND	ND	ND

Candida albicans	ND	ND	ND

TABLE 3.10: TEST FOR MINIMUM BACTERICIDAL/FUNGICIDAL CONCENTRATIONS

Key: mg/ml = Milligram per millilitre

SLOAFe, SLOA ETC = Sample codes

ND = Not detected

CONCLUSION

This study has shown that Schiff base ligand can be synthesize using 2-hydroxyaniline with acetyl chloride antipyrine (4-acyl antipyrine) as primary ligand. The melting point of the metal complexes was higher than that of the ligands but later melted and decomposed. The increases in melting point are attributed to the increase in mass of the formed complexes and thus provide evidence for complexation. Complexation of Fe(II) complex was successful using the above Schiff base ligands as shown by GCMS, FTIR, UV-VIS Spectrometric spectra interpretation.

The antimicrobial activities revealed that the complex show greater potency than the Schiff base ligand and primary ligand on the test organism due to chelation.

However, the interpretation from GCMS, UV-VIS and FTIR deduced that the octahedral geometry was proposed for the structure of the metal complex.

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Conflicts of Interest:

The authors declare that they have no conflicts of interest.

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APPENDIX

```
Created at: 10:26 23/February/2025
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Sample 1D: AURL/FTIR/LOA-Sample-01/10md11.2.1
Sample Scan: 200 scans
Backgrounds scan time: 200
scans Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 1 of 10
Save data: from 4000 cm-1 to 500 cm-1
Client Name: Consults/FTIR/LOA-sample-01#
Date: 23/02/2025
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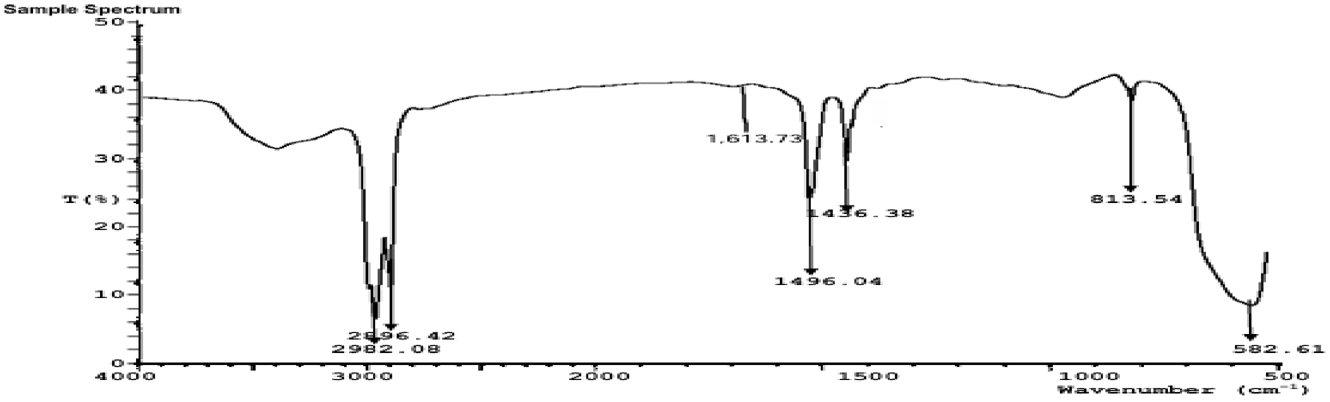

Appendix 1: Showing The Ftir Spectrum of Ligand of Acetyl Chloride Antipyrine (Loa)

```
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File Location: C\Program Files\Shmadzu\Microlab-PC\Result\Sample-03-2025-02-20T0140a2r
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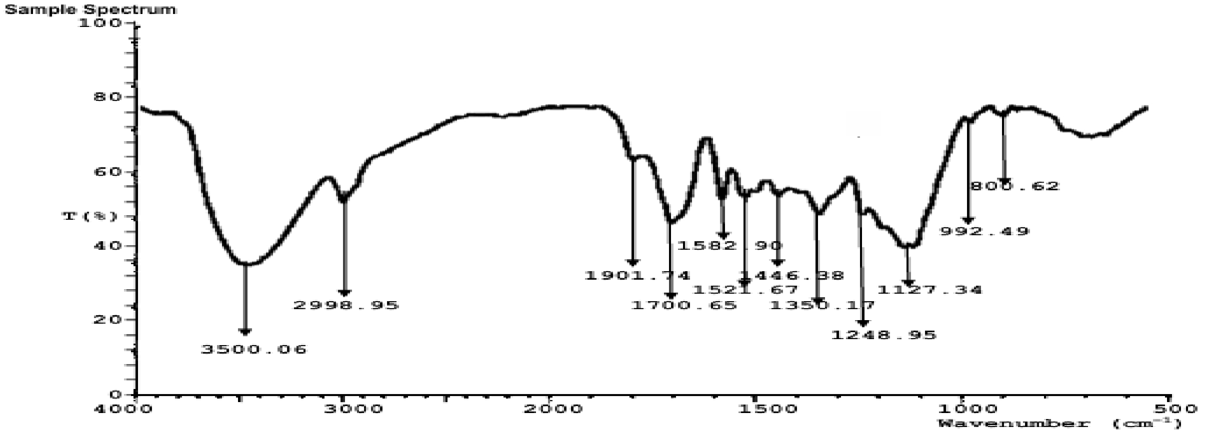
Backgrounds scan time: 200
scans Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 3 of 10
Save data: from 4000 cm-1 to 500 cm-1
Client Name: Consults/FTIR/SLOA-sample-03#
Date: 23/02/2025
```



Appendix 2: Showing The Ftir Spectrum of Schiff Base Ligand Of 2- Hydroxyl Aniline (Sloa)

```
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File Location: C\Program Files\Shmadzu\Microlab-PC\Result\Sample-05-2025-02-20T0140a2r
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Sample Scan: 200 scans
Backgrounds scan time: 200
scans Apodization: Happ-Genzel
Resolution :8
System status: Good
Full Scale 42243
Detector setting: AB_QEC-670-08
Scan Velocity-High: 40 kHz Cts
Method: Transmittance Method
Cursor Sample #: 5 of 10
Save data: from 4000 cm⁻¹ to 500 cm⁻¹
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Date: 23/02/2025
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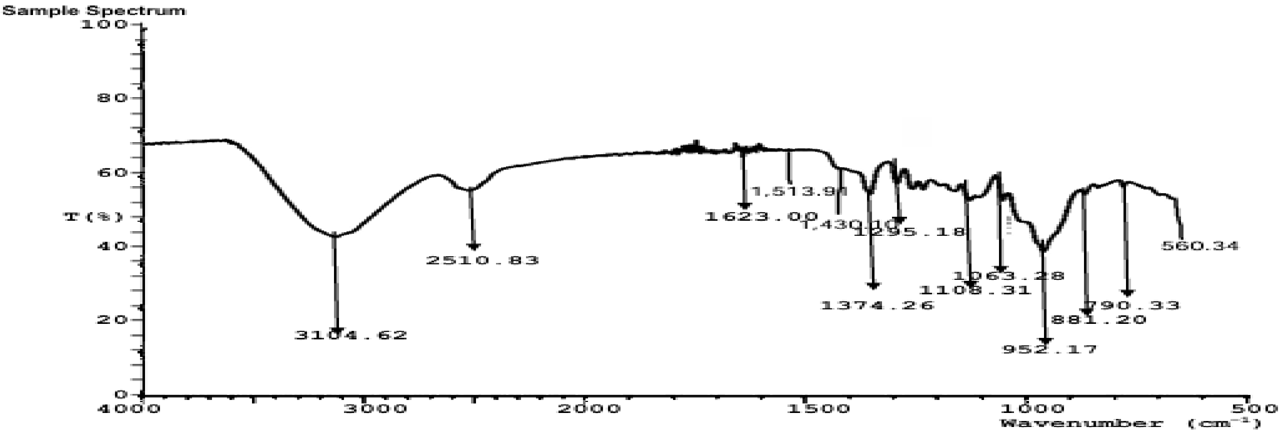
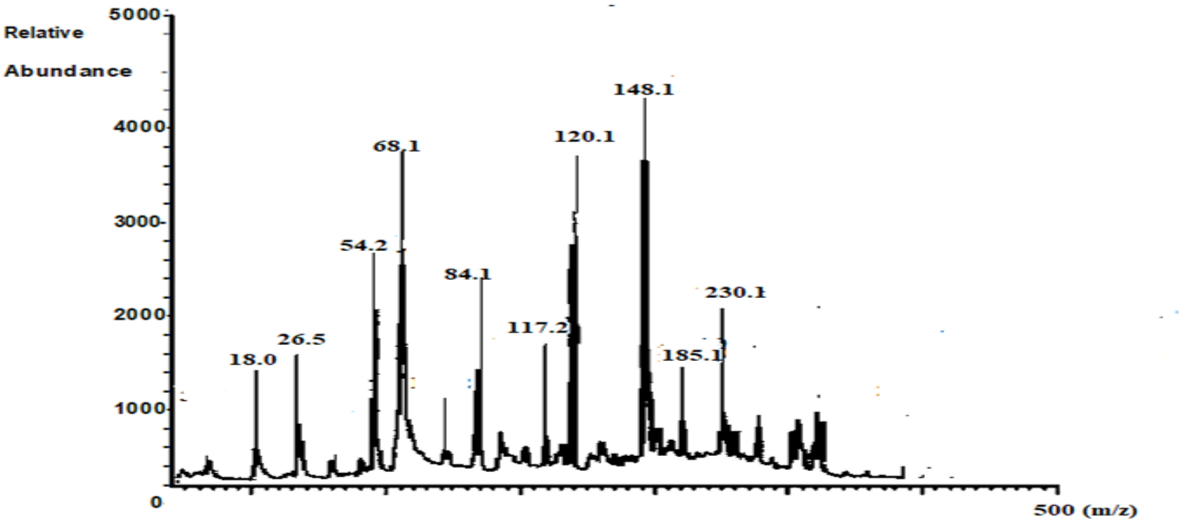


Appendix 3: Showing The Ftir Spectrum of Iron (Ii) Complex of Schiff Base Of 2- Hydroxylaniline (Sloafe)

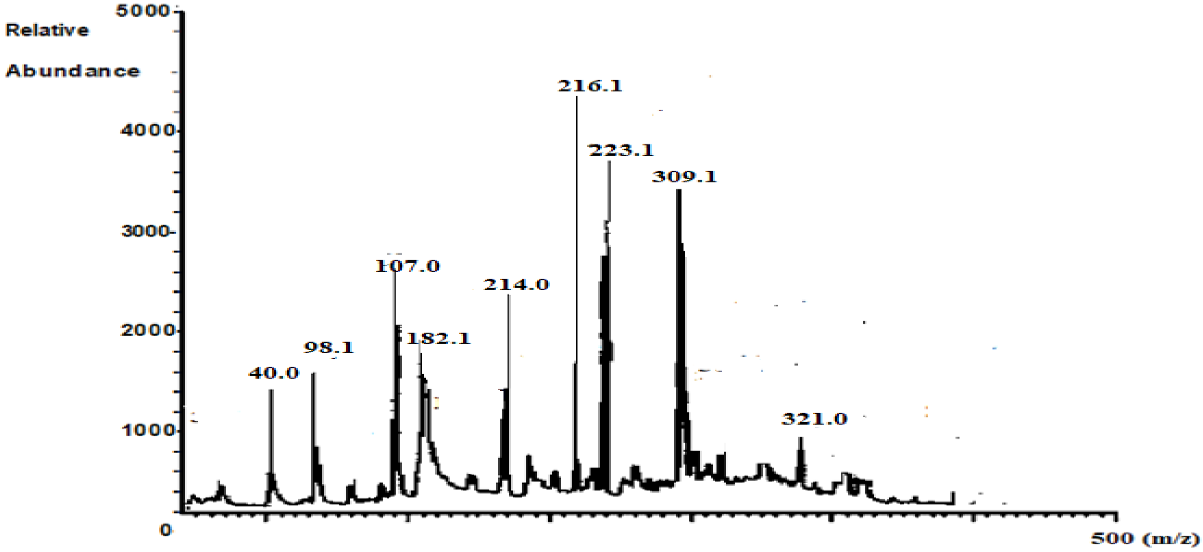
[redacted]

```
Sample ID: DCPE/CAL/GCMS/022025/119958/01       Low Mass (m/z): 0
Operator: Ewere Donatus V.                      High Mass (m/z):500
Data Path: C\varian\msdchem\data\gcms_sample_01#
Run Time: 45.00:00
Instrument Name: GCMS
Sample Name:gcms/data/LOA/sample-01#            Acquisition Date: 07/02/2025 10:23:07
Comment:
Equipment: Varian 3800/4000 GCMS                Client: Consults
ALS Vial: 1                                     Sample Multiplier: 1
Search Libraries: C:\database\NIST08            Minimum Quality: 10
Column: Agilent MS capillary column
Dimension: 30 m × 0.25 mm i.d

Carrier Gas: N2. Flow: 1.0 ml/min
Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator.autointl.e.
```



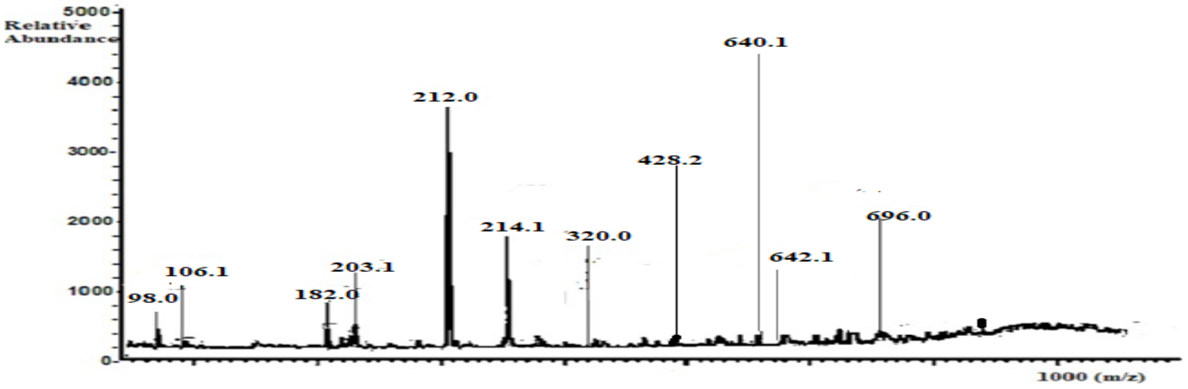
Appendix 4: Showing The Gc-Mc Spectrum of Ligand of Acetyl Chloride Antipyrine (Loa)

```
Sample ID: DCPE/CAL/GCMS/022025/119959/02       Low Mass (m/z): 0      [redacted]
Operator: Ewere Donatus V.                       High Mass (m/z):500
Data Path: C\varian\msdchem\data\gcms_sample_02#
Run Time: 45.00:00
Instrument Name: GCMS
Sample Name:gcms/data/SLOA/sample-02#            Acquisition Date: 07/02/2025 11:10:45
Comment:
Equipment: Varian 3800/4000 GCMS                 Client: Consults
ALS Vial: 1                                      Sample Multiplier: 1
Search Libraries: C:\database\NIST08             Minimum Quality: 10
Column: Agilent MS capillary column
Dimension: 30 m × 0.25 mm i.d

Carrier Gas: N2. Flow: 1.0 ml/min
Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator.autointl.e.
```

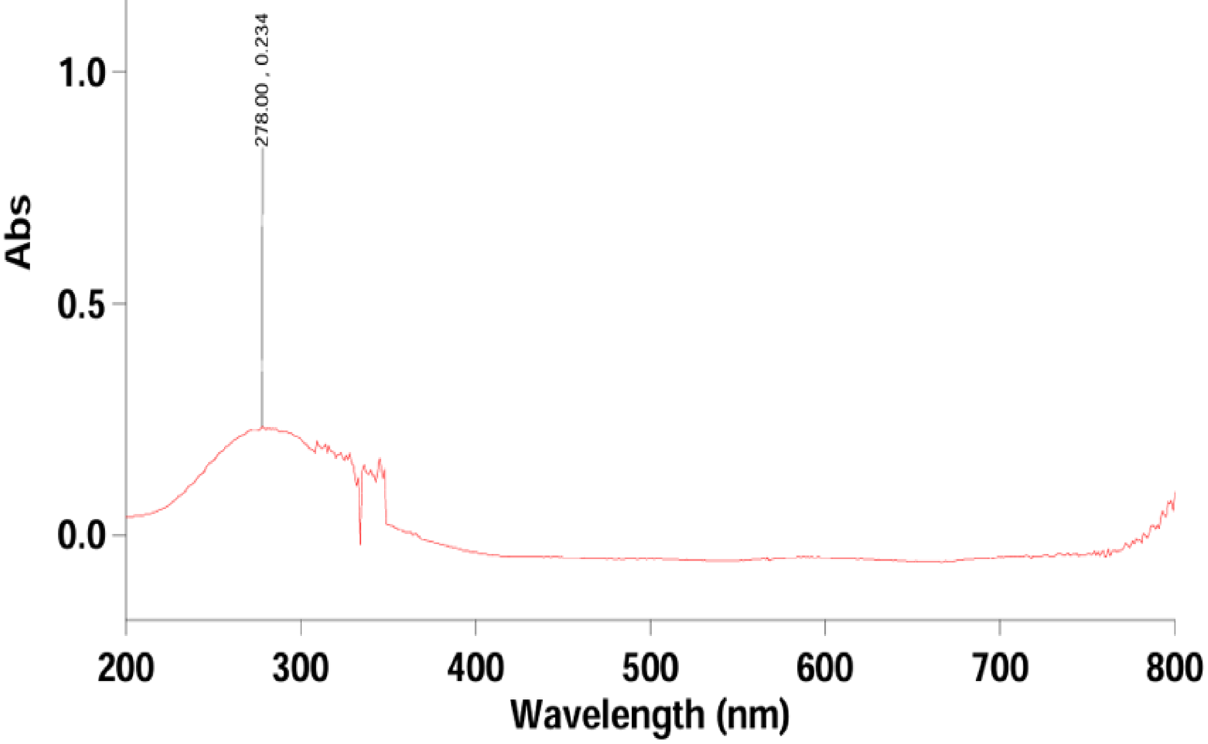


Appendix 5: Showing The Gc-Mc Spectrum of Schiff Base Ligand Of 2-Hydroxyl Aniline (Sloa)

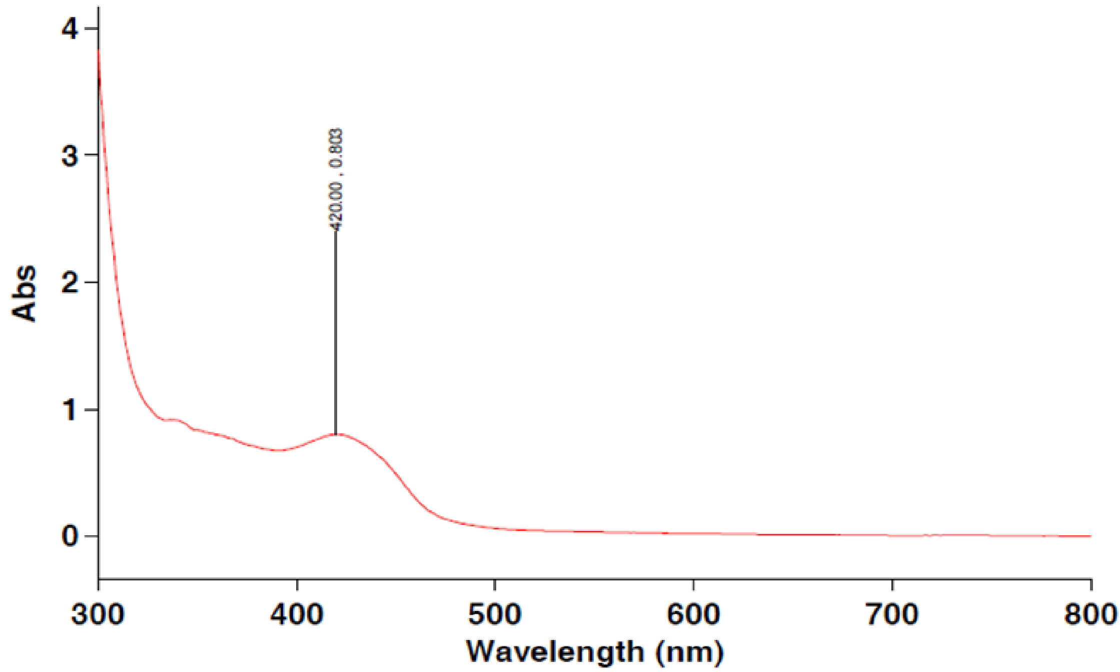
```
Sample ID: DCPE/CAL/GCMS/022025/119962/05       Low Mass (m/z): 0      [redacted]
Operator: Ewere Donatus V.                       High Mass (m/z):1000
Data Path: C\varian\msdchem\data\gcms_sample_05#
Run Time: 45.00:00
Instrument Name: GCMS
Sample Name:gcms/data/SLOA-Fe/sample-05#         Acquisition Date: 07/02/2025 13:31:43
Comment:
Equipment: Varian 3800/4000 GCMS                 Client: Consults
ALS Vial: 1                                      Sample Multiplier: 1
Search Libraries: C:\database\NIST08             Minimum Quality: 10
Column: Agilent MS capillary column
Dimension: 30 m × 0.25 mm i.d

Carrier Gas: N2. Flow: 1.0 ml/min
Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator.autointl.e.
```



Appendix 6: Showing The Gc-Mc Spectrum of Iron (Ii) Complex of Schiff Base Of 2-Hydroxyl Aniline (Sloafe)



Appendix 7: Showing The Uv-Vis Spectrum of Schiff Base Ligand Of 2-Hydroxyl Aniline (Sloa)



Appendix 8: Showing The Uv-Vis Spectrum of Iron (Ii) Complex of Schiff Base Of 2-Hydroxyl Aniline (Sloafe)