Addis Ababa University

School of Pharmacy

Department of Pharmaceutical Chemistry

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF TRIPHENYLTINBENZOATE ANILINE COMPLEX

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Pharmaceutical Analysis and Quality Assurance

By

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Abbreviations

bisTBTO	bis (tributyltin) oxide
cfu	Colony forming unit
DMSO	Dimethylsulfoxide
ev	Electron volt
IR	Infrared
LC-MS	Liquid chromatography -Mass spectrum
MIC	Minimum inhibitory concentration
MOT	Molecular orbital theory
Mp	Melting point
MS	Mass spectrum
NMR	Nuclear magnetic resonance
OT	Organotin
PPM	Parts per million
PVC	Polyvinylchloride
R_f	Retention factor
TPTCl	Triphenyltin Chloride
TPTBz	Triphenyltin benzoate
TPTBzA	Tripheyltin benzoate aniline
TBTF	Tributyltin fluoride
TBTCl	Tributyltin chloride
TBT-OAc	Tributyltin acetate
TBT	Tributyltin

TMS	Tetramethylsilane
TLC	Thin layer chromatography
WHO	World Health Organization

Abstract

Triphenyltinbenzoate and triphenyltinbenzoate aniline complex had been synthesized and characterized on the basis of infrared, mass and nuclear magnetic resonance (^{1}H and ^{13}C) spectral studies. The synthesized compounds had sharp melting point which indicated the purity of the compounds.

The antimicrobial activities of the synthesized compounds were tested against six different pathogenic microorganisms; *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes*, *Aspergillus niger and Candida ablicans* i*n vitro.* The results indicated that the synthesized compounds exhibit a higher activity against *Escherichia coli, Streptococcus pyogenes*, *Pseudomonas aeruginosa and Candida ablicans* than the starting compound. The results also indicated that the compounds did not exhibit any activity against *Aspergillus niger* at concentration as high as 200 μgml^{-1}**.**

1. Introduction

Organotin (OT) compounds are chemical compounds based on tin with hydrocarbon substituents. Organotin compounds have one or more carbon-tin covalent bonds that are responsible for the specific properties of such molecules. There are four series of organotin compounds depending on the number of carbon-tin bonds. These series are designated as mono-, di-, tri-, and tetraorganotin compounds with the general structure (WHO, 1980; Tan, 2003):

$$R_nSn\,X_{4-n}$$

Where R = an alkyl or aryl group.

Sn = the central tin atom in the oxidation state +4.

X = a singly charged anion or an anionic organic group.

n = number of alkyl or aryl group.

In the organotin compounds, R is usually a methyl, propyl, butyl, octyl or phenyl group and X is commonly chloride, fluoride, oxide, hydroxide, carboxylate, or thiolate. Monoorganotin compounds, $RSnX_3$, are known but so far have found only limited application; for example, butyltin sulfide is used as a stabilizer in poly vinyl chloride (PVC) film. Diorganotin compounds, R_2SnX_2, are chemically reactive and mostly they are used as stabilizers of PVC and as catalysts in the production of polyurethane foams (WHO, 1980).

Triorganotin compounds, R_3SnX, are the most important class of organotin chemicals. They have impressive range of applications, e.g., as pesticides, fungicides, algicides, bactericides, antifouling agents and stabilizers (Blunden *et al.*, 1985; WHO, 1980).

1.1. Physio-chemical properties of organotins

In general, the solubility of organotin compounds in water at ambient temperature is of the order of 5 to 50 mg/liter, but they are very soluble in many common organic solvents, such as alcohol, ethers, and halogenated hydrocarbons (WHO, 1980).

Tetraalkyltin compounds are colorless and the compounds of lower molecular weight are liquids at room temperature. The tetraaryltin compounds are solids. Tetraorganotin compounds possess typical covalent bonds and are stable in the presence of air and water. Tetrabutyltin, Sn $(C_4H_9)_4$ is a colorless oily liquid with a distinct odour. Tetraphenyltin, Sn $(C_6H_5)_4$, is a white crystalline powder, soluble in organic solvents and insoluble in water (WHO, 1980).

1.2. Factors that affect antimicrobial activity of organotins

The mechanism of antimicrobial activity of many organotin compounds is not fully understood. A wide range of mechanisms has, therefore, been explored (Ayoko *et al.,* 2003). The permeability of the cell affects the activity of many organotin compounds. Owing to greater lipid solubility, complexes facilitate their diffusion through the spore membrane to the site of action within the spores on the cell membranes of microbes. The variation in the effectiveness of different biocidal agents against different organisms is thus dependent on the impermeability of the cell, which in turn is affected by: (a) the molecular sizes of the substrates (Laughlin *et al.*,1985) (b) the coordination numbers about the tin atoms (Blunden *et al.*,1984; Shahzadi *et al.,* 2005) (c) the hydrophobicities of the molecules (Eng *et al.*, 1996) (d) the nucleophilicities of the anionic groups (Bonire *et al.,* 1998) (e) the lipophilic character of the metal (Joshi *et al.,* 2005) (f) the transport of the active organotin moiety to the site of the action where it is released by hydrolysis (Molloy,1989). This can be best explained on the basis of a triorganotin-ligand behavior, which dictates that the function of an ionic group is to transport the active organotin moiety to the site of the action. The ligand is displaced from tin, when the organometallic unit is bonded to the active site of the biological system, otherwise the ligand may remain bonded to tin until it reaches its receptor site. Under such circumstances, an ionic ligand may well influence the ease with which R_3SnL is transported (g) the derangement of the mitochondria of the fungi

(Aldridge,1976) (h) the inhibition of the oxidative phosphorylation function of the fungi (Ayoko *et al,* 2003). The degradative enzymes produced by the microorganisms are important in host infection, food deterioration and breakdown of organic matter. The enzyme production is here intended to mean both synthesis of the enzyme by the microorganisms and activity of the enzyme in the medium after it is produced. Since the organometal (IV) complexes inhibit the growth of microorganisms, it is assumed that the production of the enzymes is being affected; hence, the organisms are unable to utilize the food and, consequently, the growth ceases.

1.3. Hyper coordination of organotin compounds

In an ever-increasing number of papers, it is claimed that hyper coordination at the “higher” elements of the periodic table is a much more widespread phenomenon than was previously believed. Within the frame of molecular orbital theory, it is hard to see where such atoms take all the orbitals to achieve hexa-, hepta-, and octa coordination (Timosheva *et al*, 1996).

Organotin (IV) compounds readily coordinate with various donors to form hypervalent compounds. The increase in the coordination number of the central tin atom can be accomplished by a modification of a substituent at tin *via* donor→ Sn interactions.

The coordination number at tin can increase, usually beyond six, by inter- and/or intra-molecular interactions, especially in compounds where the R’ group contain a donor center. Molecular and X-ray investigations of several organotin (IV) carboxylates have revealed that the choice of structure is dictated by a subtle combination of steric and electronic effects, along with the crystal packing and hydrogen bonding effects (Kapoor *et al,* 2003).

1.4. Uses of organotins

The use of organotin has come a long way since 1960s, when tributyltin (TBT) compounds were first used as mollusicides to kill several species of fresh water snails that are intermediate hosts of the parasitic worm Schistosoma, which transmits the disease schistosomiasis to humans (Champ, 1996). The major application of organotin compounds is as heat and light stabilizer additives in PVC processing. The biocidal properties of mainly trisubstituted organotin species were used as timber preservatives. The various commercial applications gave rise to a drastic increase in the worldwide production of organotin chemicals from less than 5000 tons in 1955 up to about 50,000 tons in 1992 (Mercier *et al.*, 1994).

Organotin compounds have biocidal properties and are used; *(a)* as agricultural fungicides (triphenyltin acetate, triphenyltin hydroxide) (Davis ,1982; WHO, 1980) *(b)* as helminthicides in poultry e.g. dimethyltin dichloride (Minister of Supply and Services Canada,1993) *(c)* as nematocides (*p*-bromophenoxy triethyltin) *(d)* as herbicides (vinyl-tin compounds, e.g. trivinyltin chloride) *(e)* as rodent repellents (tributyltin chloride, triphenyltin chloride and acetate) *(f)* as bactericides and biostatics such as disinfectants for use in hospitals (tributyltin benzoate) *(g)* as ovicides (trialkyl and triaryltin chlorides in combination with DDT or pyrethrins) *(h)* furthermore, triphenyltin compounds have been suggested as insect chemosterilants (Kenaga, 1965; WHO, 1980; Gaines *et al.*, 1968) *(i)* as molluscicides (triphenyl- and tributyltin compounds) (Struffe,1968) (j) as miticides (tricyclohexyltinhydroxide) (Johnson *et al.*, 1975).

2. Objectives

2.1. General objective

- To synthesize, characterize and evaluate the antibacterial and antifungal effects of triphenyltinbenzoate and triphenyltinbenzoate aniline complex.

2.2. Specific objective

- To synthesize triphenyltinbenzoate and triphenyltinbenzoate aniline complex from the reaction of triphenyltin chloride, silver benzoate and aniline.
- To run the IR, NMR and MS spectra of the synthetic compounds to elucidate their structures.
- To determine the physical and analytical data (melting point and percentage composition) of the synthetic compounds.
- To study the antibacterial and antifungal activities of the synthetic compounds.

3. Materials and methods

3.1. Instrumentation and chemicals

3.1.1. Instrumentation

MP-D Mitaruma Rikero Kiygo electro thermal melting point apparatus was used to determine the melting points of the compounds.

Nicolet-460 spectrophotometer, Brukner Avance spectrometer and Agilent 1100 MSD LC-MS spectrometer were used to record the IR, NMR and mass spectra of the compounds respectively. Autoclave, pH meter and different glass wares were used for the antimicrobial study.

3.1.2. Chemicals

Reagent-grade triphenyltin chloride (Labmerk Chemicals LTD, India), silver nitrate (Aldrich Chemical Company, Germany), sodium benzoate (Aldrich Chemical Company, Germany), chloroform (Aldrich Chemical Company, Germany), aniline (Aldrich Chemical Company, Germany) were obtained commercially and used for the synthesis of the compounds. Muller-Hinton agar (Aldrich Chemical Company, Germany), Muller-Hinton broth (Aldrich Chemical Company, Germany), Sabouraud dextrose agar (Aldrich Chemical Company, Germany), Sabouraud dextrose broth (Aldrich Chemical Company, Germany), DMSO (Aldrich Chemical Company, Germany), barium chloride (BDH Ltd, England), sulfuric acid (BDH Ltd, England), Ketoconazole (Domina Pharmaceuticals, India), Ciprofloxcacillin (Addis Pharmaceuticals S.C., Adigrat, Ethiopia) were used for the antimicrobial study.

3.2. Synthesis of the compounds

3.2.1. Preparation of silver benzoate

First silver benzoate was prepared by the reaction of sodium benzoate and silver nitrate in water. In a typical reaction 2.40 gm (17mmol) of sodium benzoate solution was added to 2.89 gm (17mmol) of silver nitrate solution in 20ml of water under aluminum foil cover (to protect silver nitrate from light) and the white precipitate (silver benzoate) formed was filtered using suction filtration and air-dried.

3.2.2. Preparation of triphenyltinbenzoate

Triphenyltinbenzoate was prepared by the reaction of triphenyltin chloride and silver benzoate. In a typical reaction 2.4gm (10 mmol) of silver benzoate and 4.04gm (10 mmol) of triphenyltin chloride in 150 ml of chloroform were stirred for 48 hrs under aluminum cover (to protect silver benzoate from light). Then the reaction mixture was filtered to remove the formed precipitate (silver chloride). The clear solution was then evaporated to give a solid product (triphenyltin benzoate). Triphenyltin benzoate was recrystallized from chloroform. The formation of the complex was detected by TLC and confirmed from the spectral data recorded.

3.2.3. Preparation of triphenyltinbenzoate aniline complex

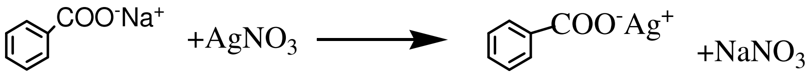
The complex, triphenyltinbenzoate aniline was prepared by adding aniline to triphenyltinbenzoate at room temperature. In atypical reaction 1gm (2.12mmol) of triphenyltinbenzoate and 0.395gm (4.24mmol) of aniline in 30 ml of chloroform were left at room temperature for 24 hrs and after 24 hrs the product was recrystallized from Chloroform and petroleum ether (in ratio of 3:1). The formation of the complex was detected by TLC and confirmed from the spectral data recorded.

3.2.4. Thin-layer chromatography (TLC)

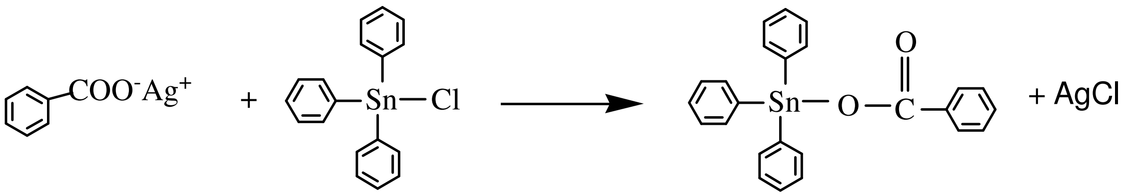
Thin-layer chromatography was used to detect the formation of the compounds. Precoated silica gel 0.25 mm thickness plates on aluminum foil E. Merck was used for the chromatography and Iodine vapor was used to detect the spots.

3.3. Chemical reactions

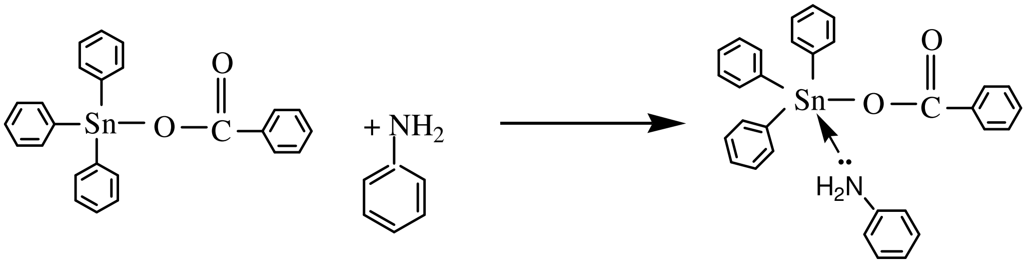
1.Preparation of silver benzoate



2.Preparation of triphenyltin benzoate



3.Preparation of triphenyltin benzoate aniline complex



3.4. Antimicrobial activity

The synthetic compounds and triphenyltin chloride were screened against antibacterial and antifungal activities *in vitro*. The antibacterial activity of the synthesized compounds against *Escherichia coli (ATCC25922)*, *Pseudomonas aeruginosa (ATCC27853), Staphylococcus aureus (ATCC25923),* and *Streptococcus pyogenes (clinical isolate),* and the antifungal activity against *Aspergilus niger (clinical isolate)* and *Candida ablicans (clinical isolate)* were screened using the agar well diffusion method. Ciprofloxcacillin was used as standard drug against bacteria and ketokonazole against fungi.

3.4.1. Qualitative antibacterial activity test

The qualitative antibacterial activity of the compounds was determined using agar diffusion method. Solutions of four different concentrations (200µg/ml, 20µg/ml' 2µg/ml, 0.2µg/ml) of the compounds [triphenyltin chloride ($C_{18}H_{15}SnCl$), triphenyltin benzoate ($C_{25}H_{20}SnO_2$) and triphenyltinbenzoate aniline complex ($C_{31}H_{27}SnO_2N$)] were prepared in dimethylsulfoxide (DMSO) from 400µg/ml stock solution.

Then Muller–Hinton agar was prepared according to the manufacturer's instruction and autoclaved at 121°C for 15 minutes. Then 20 ml of the media was transferred in to previously sterilized peteridish and allowed to solidify at room temperature. Two to eight hours old bacterial inoculums containing 10^8 cfu/ml were spread on the surface of Muller-Hinton agar with the help of a sterile cotton swab. Then four wells were dug in the media with the help of a sterile cork borer

(5mm of internal diameter) with centers at least 24 mm apart and two wells in each plate were then filled with 100µl of test solutions. Other wells were supplemented with DMSO and reference antibacterial drug (negative control). Then the plates were left undisturbed on the bench for 2 hours at room temperature. The plates were subsequently incubated at 35^0C temperature for 24h. The activity was determined by measuring the diameter of zones showing complete inhibition in millimeters (mm). Growth inhibition results are collected in Table 6. Triplicate measurements were done.

3.4.2. Quantitative antibacterial test

3.4.2.1. Minimal Inhibitory Concentration (MIC)

The quantitative antibacterial results are reported in terms of minimum inhibitory concentrations (MICs).

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2006).

3.4.2.2. Broth Tube Dilution Method

The tube dilution test is the standard method for determining levels of minimum inhibition concentration. Serial dilutions of the antibiotic are made in a liquid medium, which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). At this dilution the test compound is bacteriostatic. Although the tube dilution test is fairly precise, the test is laborious because serial dilutions of the antimicrobial compound must be made and only one organism can be tested in each series of dilutions (Rollins and Joseph, 2000).

3.4.2.3. Method

Serial dilutions of the test compounds were prepared in test tubes containing Muller-Hinton broth as a diluent. Each bacterium was inoculated into different tube accordingly containing the diluted compounds and then incubated at 35°C for 24h. The tubes were then examined for the presence or absence of growth considering turbidity as a criterion. The lowest concentration that showed inhibition was further diluted to different concentrations in order to determine the MIC. The highest dilution in each series that showed no growth (turbidity) of test organism was considered to be the MIC of that organism.

Sterile capped test tubes 1 through 9 were numbered and then 2ml of test solution of (200μg/ml prepared from stock solution) was added to the first sterilized test tube. Muller-Hinton Broth (1ml) was added to the rest of the rest tubes. Then 1ml of the test solution was transferred to the second tube. The contents of this tube were mixed thoroughly and using a different pipette 1 ml of the solution was transferred to the third tube. The dilution continued in the same manner to test tube 8, being careful between pipettes to prevent exchange of carryover of test solutions. One ml of the test solution was then removed from test tube 8 and discarded. The nineth tube which served as a negative control, received no test solution.

3.4.2.4. Preparation of the inoculums

The inoculums were adjusted so that 10^8 cfu/ml were applied to the tubes. The desired inoculums were prepared by comparison with a 0.5 McFarland standard. At least four morphologically similar colonies were taken by a sterile loop. And transferred for growth into 5.0 ml of Mueller-Hinton broth and incubated with shaking at 35-37℃ until the visible turbidity was equal to or greater than the 0.5 McFarland standard. The density of the organism suspensions prepared were adjusted equal to that of the 0.5 McFarland standard by adding sterile distilled water. Suspensions contain 10^8 cfu/ml.

3.4.2.5. Preparation of the McFarland standard

0.5 ml of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% v/v) with constant stirring. And then the prepared standard was distributed into screw cap tubes of the same size and with the same volume as those used in growing the broth cultures. The tubes were sealed tightly to prevent loss by evaporation and stored at room temperature protected from light. They were vigorously agitated on a vortex mixer before use to achieve the turbidity standard.

3.4.2.6. Inoculation

1.0 ml of the diluted culture suspensions were added to each of the tubes. And then the contents of the test tubes were mixed thoroughly. The final concentration of the test compounds were now one-half of the original concentration in each tube i.e. the concentrations now become 100, 50, 25 ,12.5, 6.25, 3.13, 1.56, 0.78 μg/ml .Then the test tubes were incubated at 35^{o}C overnight. After 24 hours of incubation the tubes are examined for visible signs of bacterial growth. The lowest concentration that showed inhibition was further diluted to different concentrations in order to determine the MIC. The highest dilution without growth was determined to be the minimal inhibitory concentration (MIC).

3.4.3. Qualitative antifungal activity test

For qualitative antifungal test, Sabouraud dextrose agar was used. Similar procedures were followed to determine the zone of inhibition except the incubation temperature (25^{o}C) and the incubation time (48 hours).

3.4.4. Quantitative antifungal test

3.4.4.1. The minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations (MICs) were determined in Sabouraud dextrose broth by broth dilution method. Similar procedure was used to determine the minimum inhibitory concentrations except the incubation temperature (25^{o}C) and the incubation time (48 hours).

4. Results and discussion

4.1. Melting point and percentage yield

The melting points were determined in a capillary tube using an MP-D Mitaruma Rikero Kiygo electrothermal melting point apparatus. All the synthesized compounds showed a sharp melting point which indicated their purity. The melting point (mp) data of the synthetic compounds are reported in Table 1. The reactions gave high yield of the products.

Table 1: Melting point and percentage yield of the investigated compounds

Compound	M.P. (^{0}C)	Yield (%)
Triphenyltinbenzoate	79-80	89.9
Triphenyltinbenzoateaniline	101-103	74.6

Elemental analysis of the compounds was not done because of the lack of instrument to do the analysis.

4.2. Thin-layer chromatography (TLC)

The difference in the R_f values of the synthesized compounds and the precursor compound gave a preliminary information for the formation of the compounds, which was confirmed by the recorded spectral data.

Table 2: TLC results of the compounds

	R_f values	
	Benzene-Chloroform-Methanol = 10:9:0.5	Benzene-Chloroform-Methanol =14:11:1
Triphenyltin chloride	0.4	*
Triphenyltin benzoate	0.48	0.57
Triphenyltinbenzoate aniline complex	*	0.48

*** TLC had not been run with**

4.3. Spectroscopic analysis of the studied compounds

4.3.1. Infrared Spectroscopy

IR spectra in the 4000-335.7 cm^{-1} range were recorded. IR spectra were recorded with a Nicolet-460 spectrophotometer using KBr discs. The characteristic vibrational frequencies were identified by comparing the spectra of the synthetic compounds with their precursor (triphenyltin chloride). The formation of triphenyltinbenzoate was confirmed by the presence of bands at 1626.06 cm^{-1}(strong–O-CO-), 617.18-661.27cm^{-1} (weak, Sn−O) and 1069.74cm^{-1}(weak, Sn-O-CO) respectively. The IR spectrum of triphenyltinbenzoate aniline complex showed additional characteristic peak at 2928.14 cm^{-1} which could be due to (N-H) vibrations. The appearance of medium intensity bands in the range 617-450 cm^{-1} due to (Sn−C) and (Sn−O) vibrations further confirms the formation of the compounds.

4.3.2. 1H NMR Spectroscopy

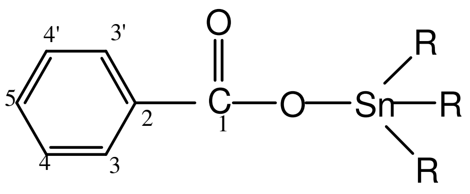
^{1}H and ^{13}C NMR spectra were recorded with a Brukner Avance spectrometer operating at 400 MHz. The spectra were acquired at room temperature (298^o K). The chemical shifts are reported in parts per million (ppm) with respect to the reference tetramethylsilane (TMS) for ^{1}H and ^{13}C NMR. Deutrated chloroform was used as a solvent.

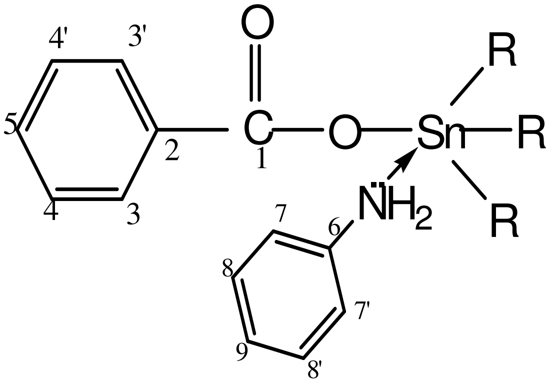
^{1}H NMR spectral data of reported compounds are given in Table 3. Different protons were assigned on the basis of their multiplicity and intensity patterns and literature values. The ^{1}H NMR spectrum of triphenyltinbenzoate showed a multiplate peak at δ 7.4-7.6 due to the effect of the carbonyl group. The ^{1}H NMR spectrum of triphenyltinbenzoate aniline complex exhibited NH_2 protons at δ 4.2-4.45ppm. The downfield shift of the NH_2 signal form δ 3.55 (in the free aniline) to *δ4.2-4.45* (in tripnenyltinaniline complex) is probably due to a transfer of charge towards the more electropositive tin. The comparative study of the ^{1}H NMR spectra of the starting materials and the complexes suggested that the proposed complexation to the tin atom was achieved.

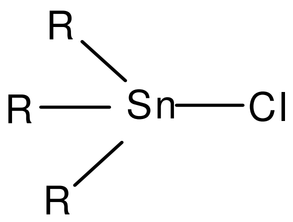
Table 3: ^{1}H NMR data for the test compounds

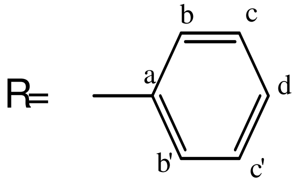
Proton No.	Compound		
	TPTCl	TPTBz	TPTBzA
3,3’	-	8.12-8.17(d)	8.1-8.3(m)
4,4’	-	7.35-7.6(m)	7.4-7.6(m)
5	-	7.35-7.6(m)	7.4-7.6(m)
7,7’	-	-	7.7-7.8(m)
8,8’	-	-	7.4-7.6(m)
9	-	-	7.4-7.6(m)
NH_2	-	-	4.2- 4.45
b,b’	7.64-7.7(m)	7.78-7.82(m)	7.8-7.9(m)
c,c’	7.43-7.48(m)	7.35-7.6(m)	7.4-7.6(m)
d	7.43-7.48(m)	7.35-7.6(m)	7.4-7.6(m)

Chemical shift (δ) in ppm,
Multiplicity is given as s = singlet, doublet, t = triplet, m = multiplet









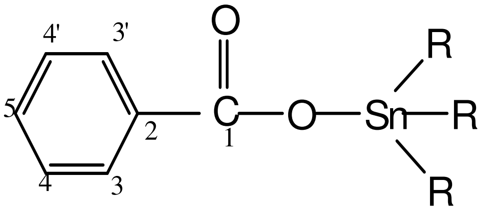
4.3.3. 13C-NMR Spectroscopy

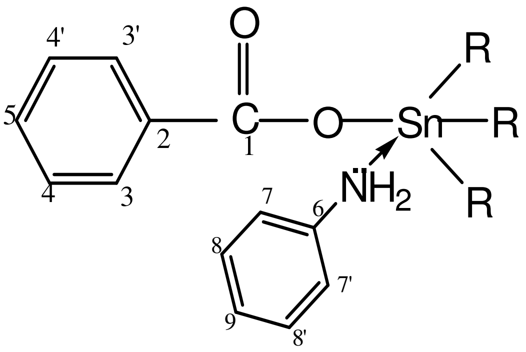
^{13}C NMR spectral data for all compounds were recorded in deuterated chloroform. The ^{13}C NMR spectral data for the studied compounds are reported in Table 4. A certain overlap of the values was observed for ^{13}CNMR signals. Resonances were assigned by comparing the experimental chemical shifts with those in literature values (Pretsch *et al*, 2000).

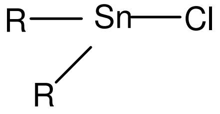
Table 4: ^{13}C NMR data for the studied compounds

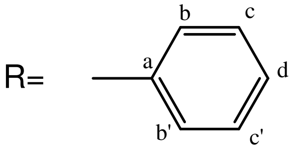
Carbon No.	Compound		
	TPTCl	TPTBz	TPTBzA
1	-	172.83	
2	-	130.73	130.89
3,3’	-	130.2	130.65
4,4’	-	128.25	128.81
5	-	132.76	132.57
6	-	-	*
7,7’	-	-	128.16
8,8’	-	-	129.24
9	-	-	128.61
A	137.34	137.25	137.18
b,b’	135.93	136.78	132.67
c,c’	128.89	128.7	128.8
D	130.6	130.28	130.16

*Not observed



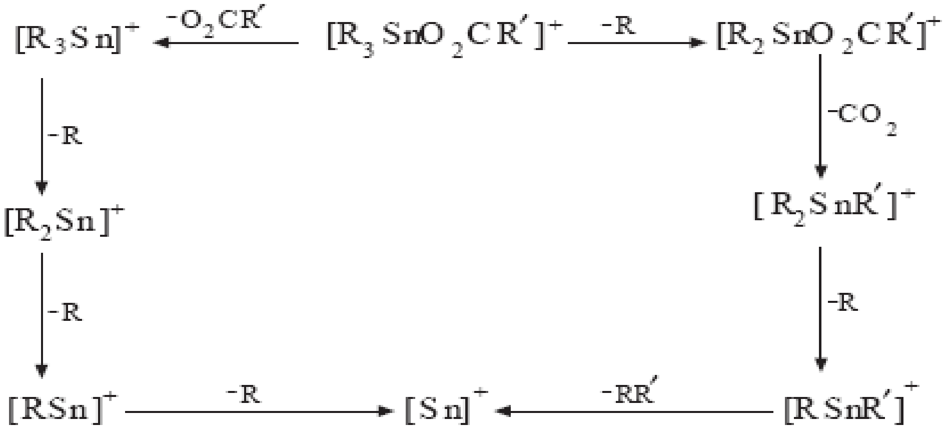






4.3.4. Mass Spectroscopy

Mass spectrum was determined on an Agilent 1100 MSD LC-MS at Indian Institute of Chemical Biology. The main fragment ions observed in the mass spectra of compounds are listed in Table 5. The fragmentation behavior is described in Scheme 1. The fragmented ions are in good agreement with the expected structures of the compounds. The mass spectra are dominated by the daughter ions that have lost one or more phenyl groups. Sodium matrix-assisted laser desorption ionization (MADLI) mass spectra was used in the ms analysis of the compounds. If there are traces of metal salts in the sample, clusters of the type [M+ metal cation]$^+$ occurs in the positive ionization mass spectra (Pretsch *et al*, 2000). The molecular ion peak of TPTBz appeared at m/z 495 (M+23). The molecular ion peak in TPTBzA was not observed.



Scheme 1: General fragmentation pattern for triorganotin carboxylates (Shahid *et al*, 2003)

Table 5: Relative abundance (%) of common ions observed

Fragment ion	TPTBz m/z (%)	TPTBzA m/z (%)
$[PhSn]^+$	197(20)	197(n.o)
Sn^+	120(3)	120(n.o)
$[Ph_3Sn]^+$	350 (100)	350(100)
$[Ph_3SnCO_2]^+$	394.99(21)	395.11(6)
$[Ph_3SnPh]^+$	428(n.o)	428(4)
$[Ph_3SnCO_2Ph]^+$	(472)n.o	-
$[Ph_3SnCO_2Ph+Na]^+$	495(15)	495(5)
$[Ph_3SnCO_2PhNH_2Ph]^+$	-	565(n.o)

n. o. = not observed

4.4. Antimicrobial activity of the compounds

The results presented in Table 6 and Table 7 clearly illustrated that TPTCl, TPTBz and TPTBzA had antimicrobial activity against the tested microorganisms. When the antimicrobial activity of the compounds was compared with that of the standard drugs used, it was found to be less potent. The negative control and dimethyl sulfoxide showed no antimicrobial activity against the tested organisms.

The result also revealed that among the six micro-organisms against which the antimicrobial activity was tested, *Escherichia coli* was observed to be the most susceptible microbe to the three tested compounds.

Table 6: Diameter of inhibition zone (mm) of the compounds against bacteria

Name of compound	Type of bacteria	Concentration µgml^{-1}			
		200	20	2	0.2
TPTCl	*Escherichia coli*	20.5±0.06	15.1±0.06	-	-
	Staphylococcus aureus	18.27±0.15	15.1±0.06	-	-
	Streptococcus pyogenes	15±0.06	12.2±0.1	-	-
	Pseudomonas aeruginosa	14.5±0.06	6.17±0.06	-	-
TPTBz	*Escherichia coli*	24.5±0.1	15.5±0.17	-	-
	Staphylococcus aureus	21.5±0.1	14.16±0.1	-	-
	Streptococcus pyogenes	21.5±0.1	16.5±0.1	-	-
	Pseudomonas aeruginosa	11.13±0.15	9.5±0.1	-	-
TPTBzA	*Escherichia coli*	25.5±0.11	16.7±0.17	-	-
	Staphylococcus aureus	24.17±0.1	13.2±0.1	-	-
	Streptococcus pyogenes	19.5±.01	14.5±0.1	-	-
	Pseudomonas aeruginosa	11.5±0.15	9.5±0.1	-	-
Standard drug (ciproflocacillin, 0.3%)	*Escherichia coli*	29.87±0.15			
	Staphylococcus aureus	29.26±0.15			
	Streptococcus pyogenes	25.57±0.15			
	Pseudomonas aeruginosa	33.28±0.06			

Key:TPTCl Triphenyltin chloride; TPTBz Triphenyltinbenzoate; TPTBzA Triphenyltinbenzoate aniline; (-) score was recorded for no activity. All results are mean value ± SD, DMSO did not show any zone of inhibition.

Table 7: Diameter of inhibition zone (mm) of the compounds fungi

Name of compound	Type of Fungi	Concentration µgml^{-1}			
		200	20	2	0.2
TPTCl	*Aspergillus niger*	-	-	-	-
	Candida ablicans	14±0.1	7.2±0.1	-	-
TPTBz	*Aspergillus niger*	-	-	-	-
	Candida ablicans	19±0.1	12±0.1	-	-
TPTBzA	*Aspergillus niger*	-	-	-	-
	Candida ablicans	20±0.1	13.5±0.1	-	-
Standard drug (ketokonazole 0.3%)	*Aspergillus niger*	11.5±0.1			
	Candida ablicans	23.17±0.1			

Key = TPTCl Triphenyltin chloride; TPTBz Triphenyltinbenzoate; TPTBzA Triphenyltinbenzoate aniline; (-) score was recorded for no activity; All results are mean value ± SD (standard deviation), DMSO did not show any zone of inhibition.

Table 8: MIC ($\mu g ml^{-1}$) of the compounds against bacteria and fungi

Compound	*E. coli*	*S. aureus*	*S. pyogenes*	*P. aeruginosa*	*C. ablicans*
TPTCl	12.5	7.5	20	10	10
TPTBz	7.5	7.5	7.5	10	5
TPTBzA	7.5	7.5	7.5	7.5	5

5. Conclusion

All the studied compounds showed significant activity against the tested bacteria and fungi. Based on the magnitude of diameters of the inhibition zones and the corresponding MIC values, it was seen that TPTBz and TPTBzA were relatively more active against the tested organisms than TPTCl. TPTCl, TPTBz and TPTBzA showed no activity at 200 µgml^{-1} against *A. niger.* TPTBz and TPTBzA showed equal activity against *E. coli, S. aureus, S. pyogenes* but TPTBz had lower activity than TPTBzA against *P. aeruginosa.* TPTBz and TPTBzA caused better inhibition of fungal growth than TPTCl.

Generally all the compounds showed markedly lower antifungal and antibacterial activity than the standard drugs used. The antimicrobial activity of the tested compounds suggested that the compounds could be used as potential antibacterial and antifungal agents against the tested organisms.

6. Recommendation

The following recommendations are given for further studies on the compounds evaluated.

- The results obtained here suggest that these compounds need to be studied further for their applications as antimicrobials.
- The Anticancer and anti-TB activities of many organotin compounds have been reported and thus the synthesized compounds can also be tested for their anticancer and anti-TB activities.

7. References

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8. Annex

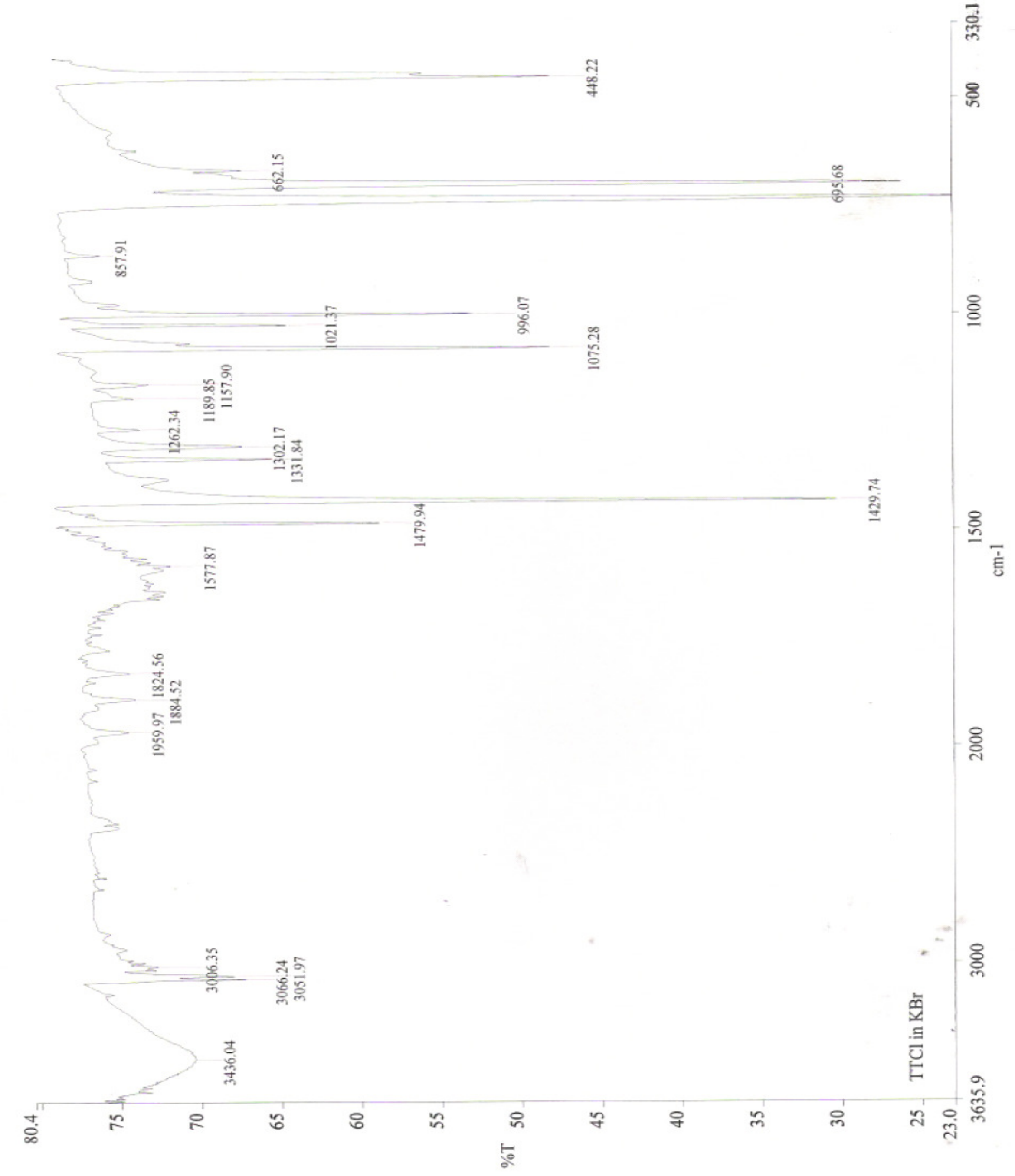


Fig 1. IR spectrum of triphenyltin chloride

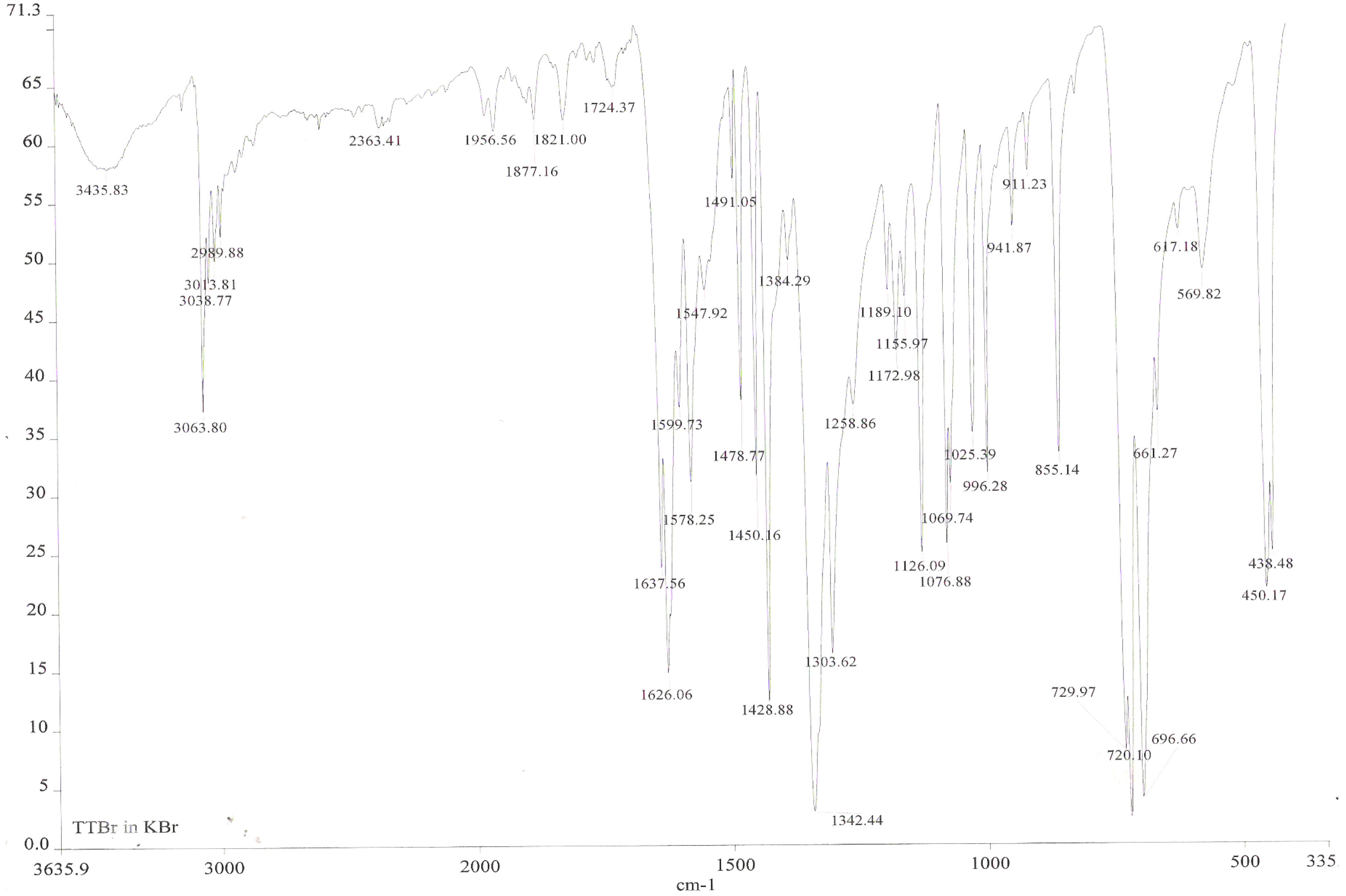


Fig 2. IR spectrum of triphenyltin benzoate

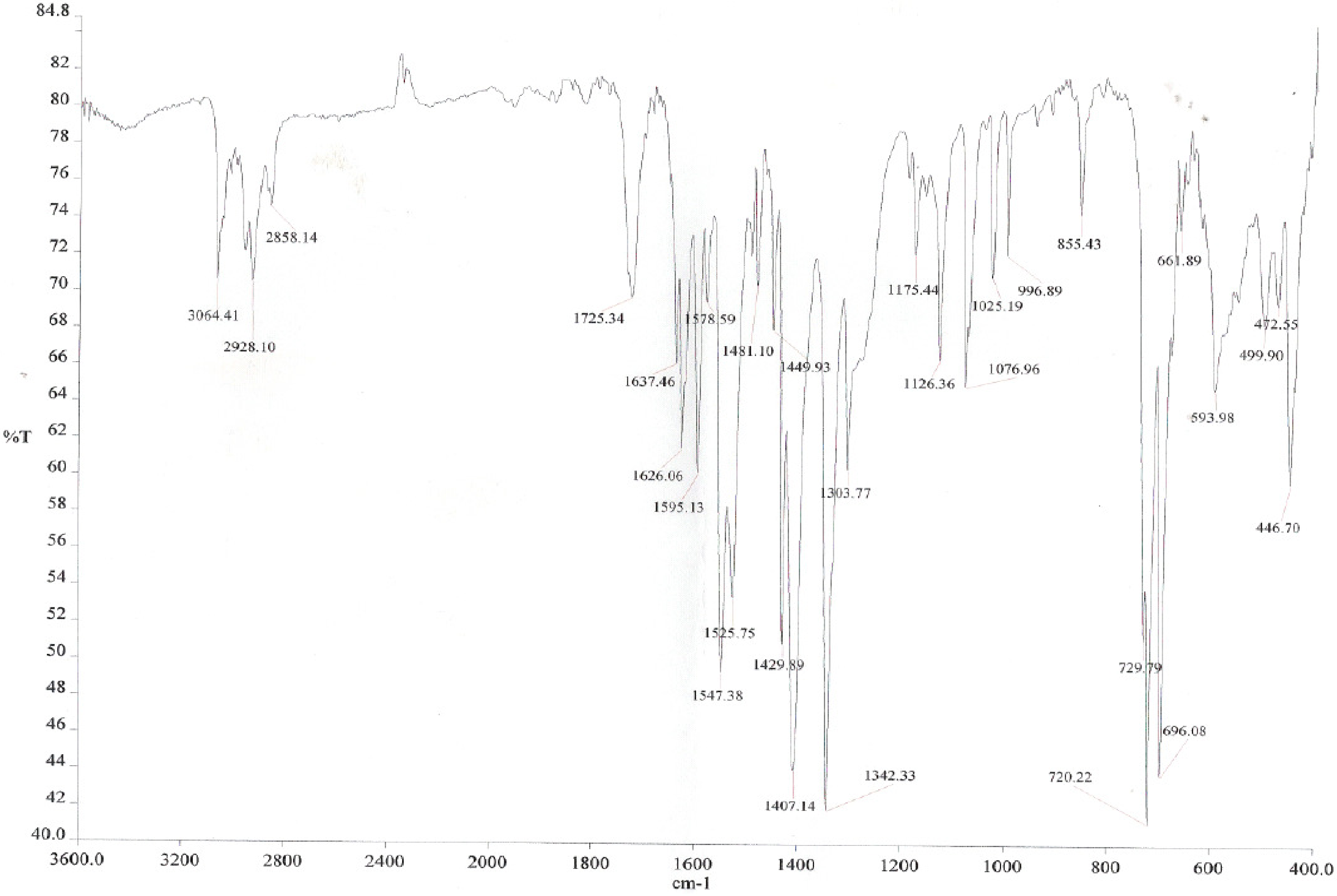


Fig 3. IR spectrum of triphenyltin benzoate aniline complex

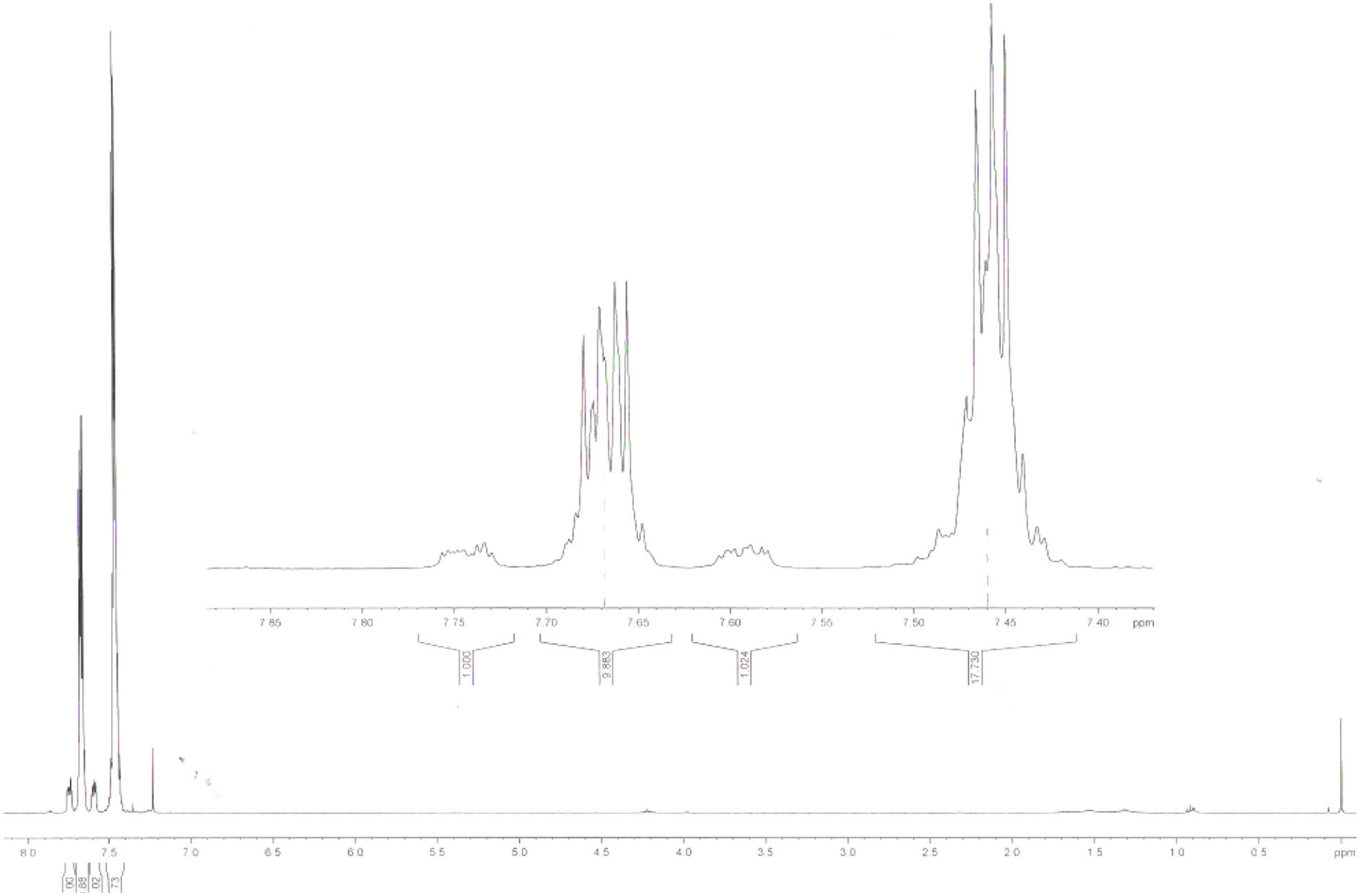


Fig 4. ^{1}H NMR spectrum of triphenyltin chloride

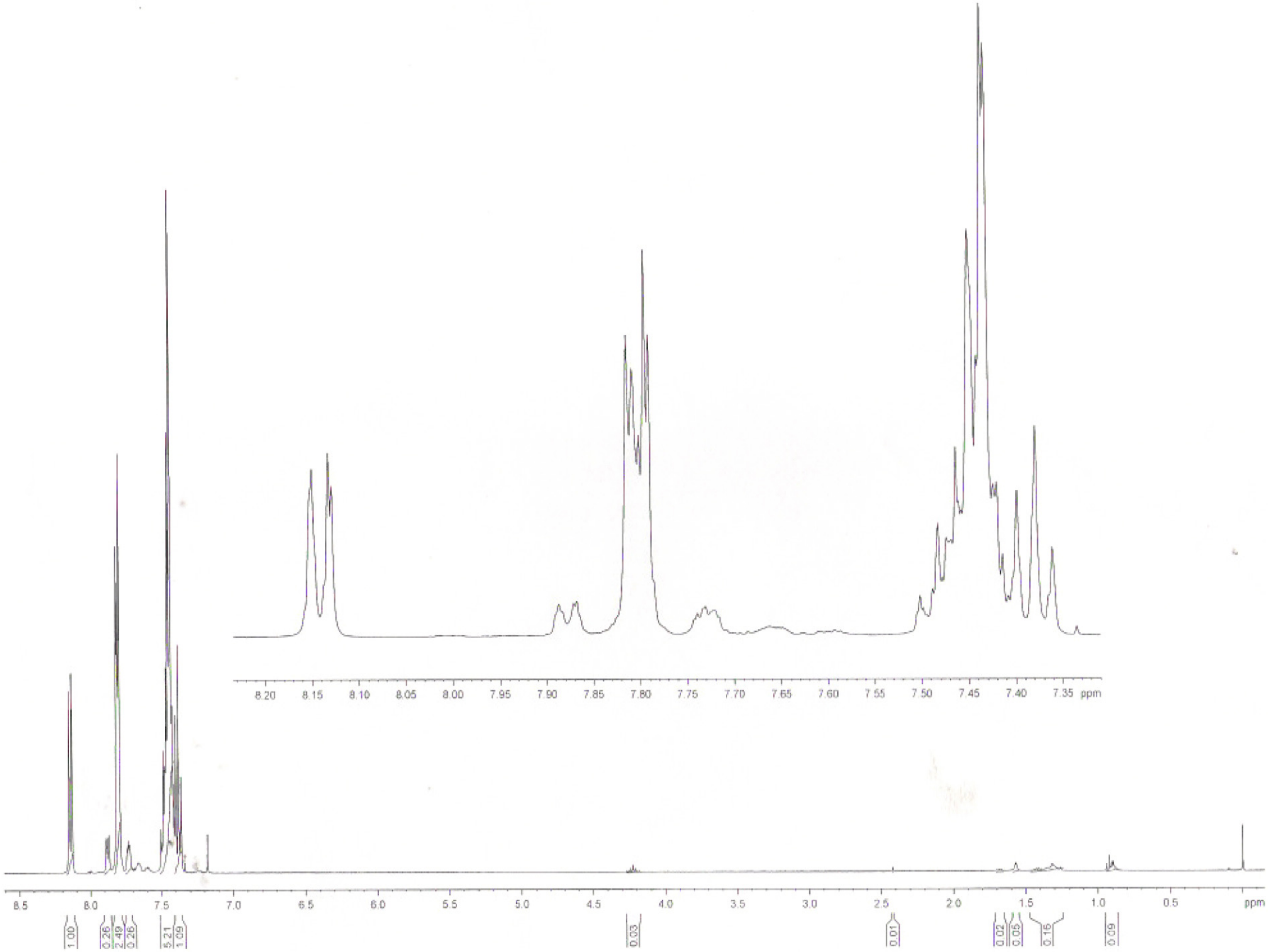


Fig 5. ^{1}H NMR spectrum of triphenyltinbenzoate

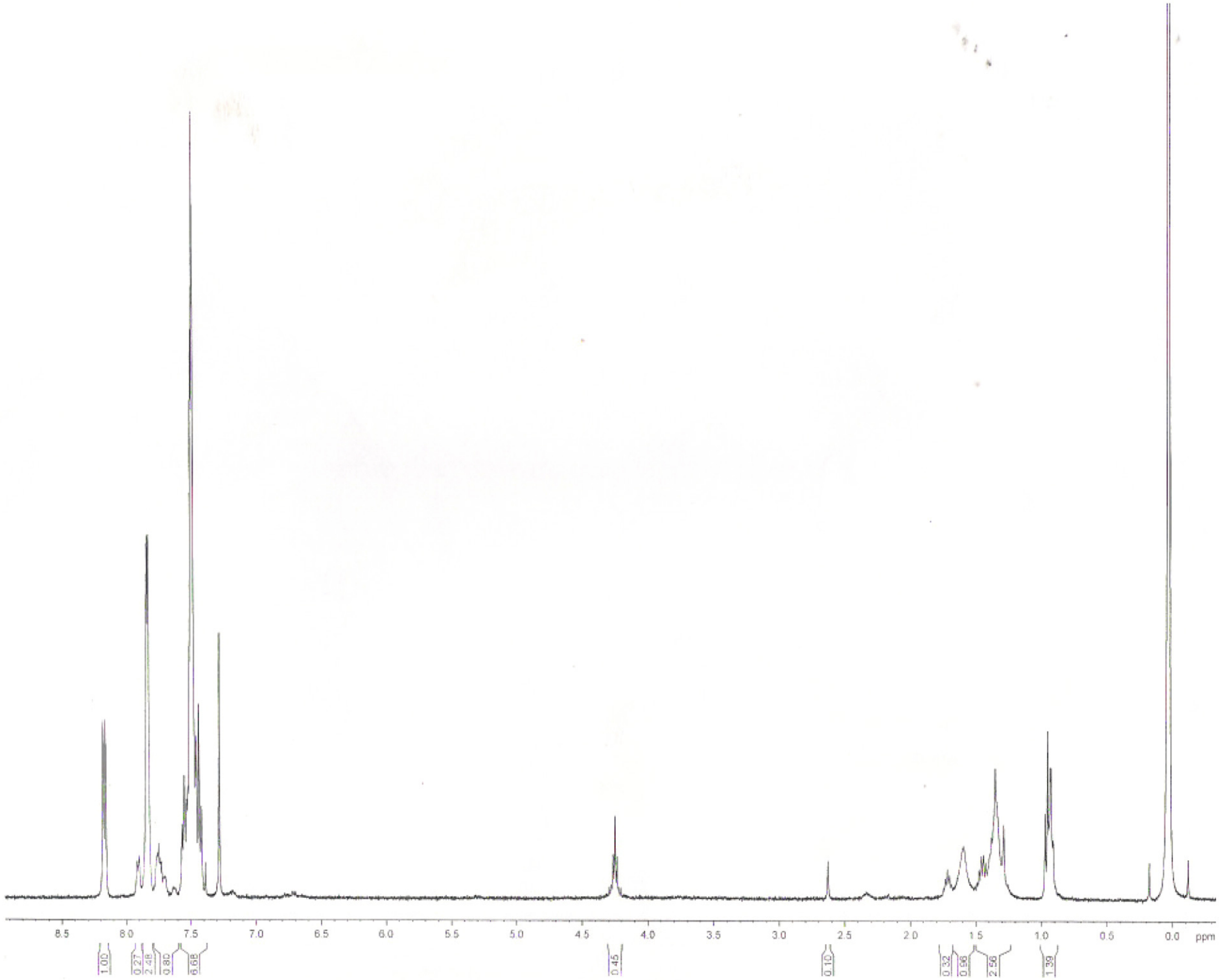
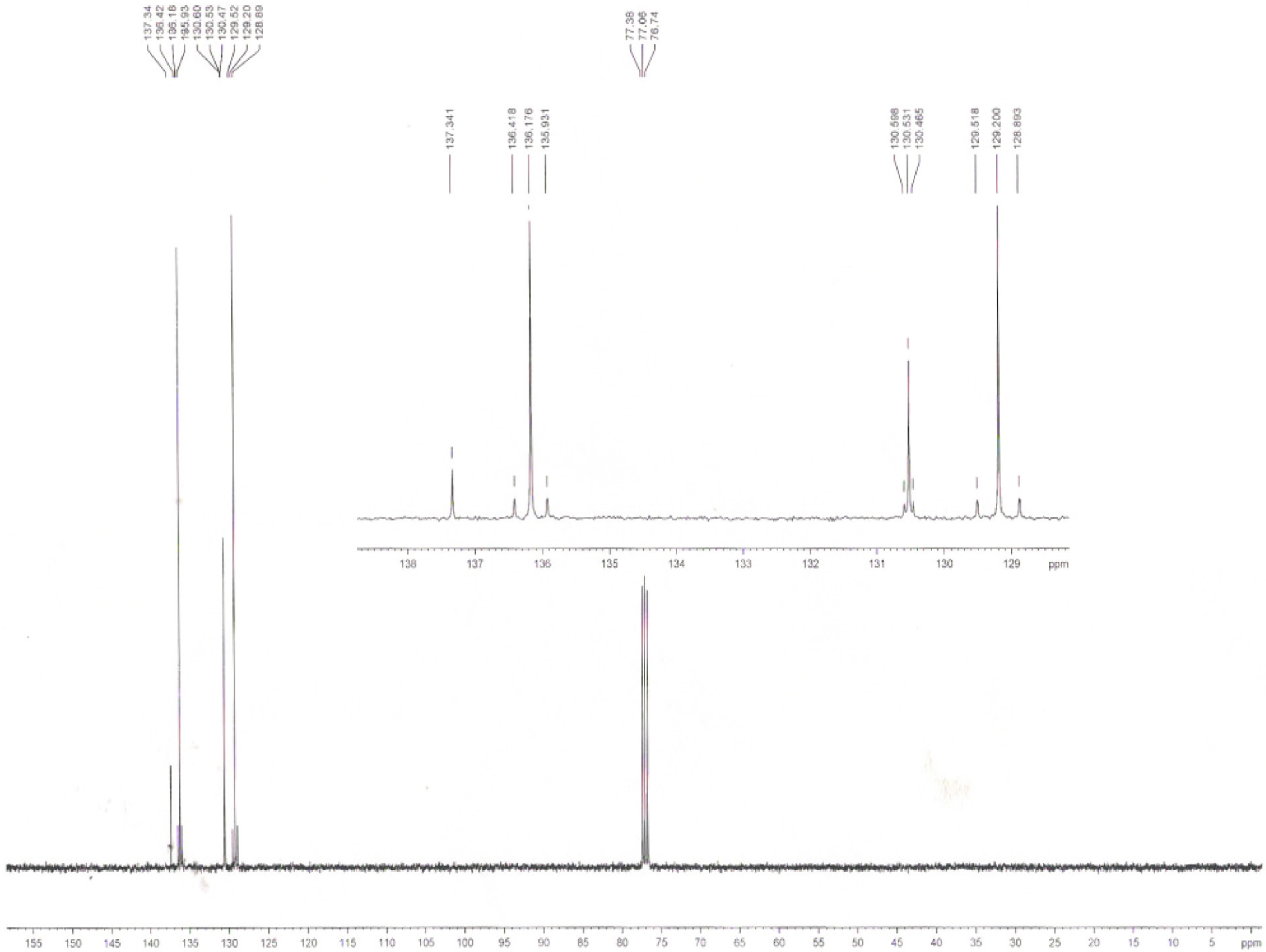


Fig 6. ^{1}H NMR spectrum of triphenyltinbenzoate aniline



`Fig 7. **^{13}C** -NMR spectrum of triphenyltin chloride

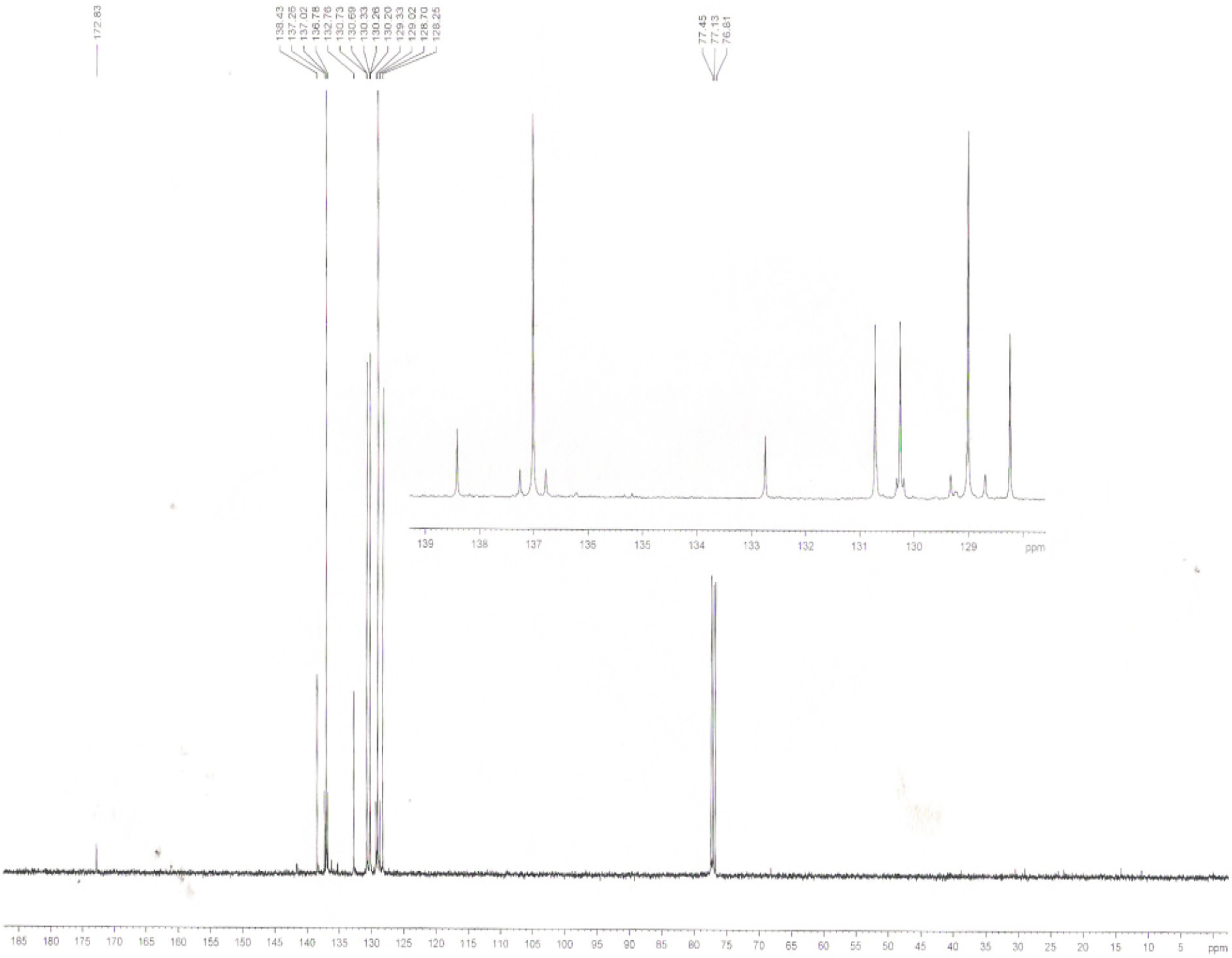


Fig 8. ^{13}C-NMR spectrum of triphenyltinbenzoate

Fig 9. ^{13}C-NMR spectrum of triphenyltinbenzoate aniline

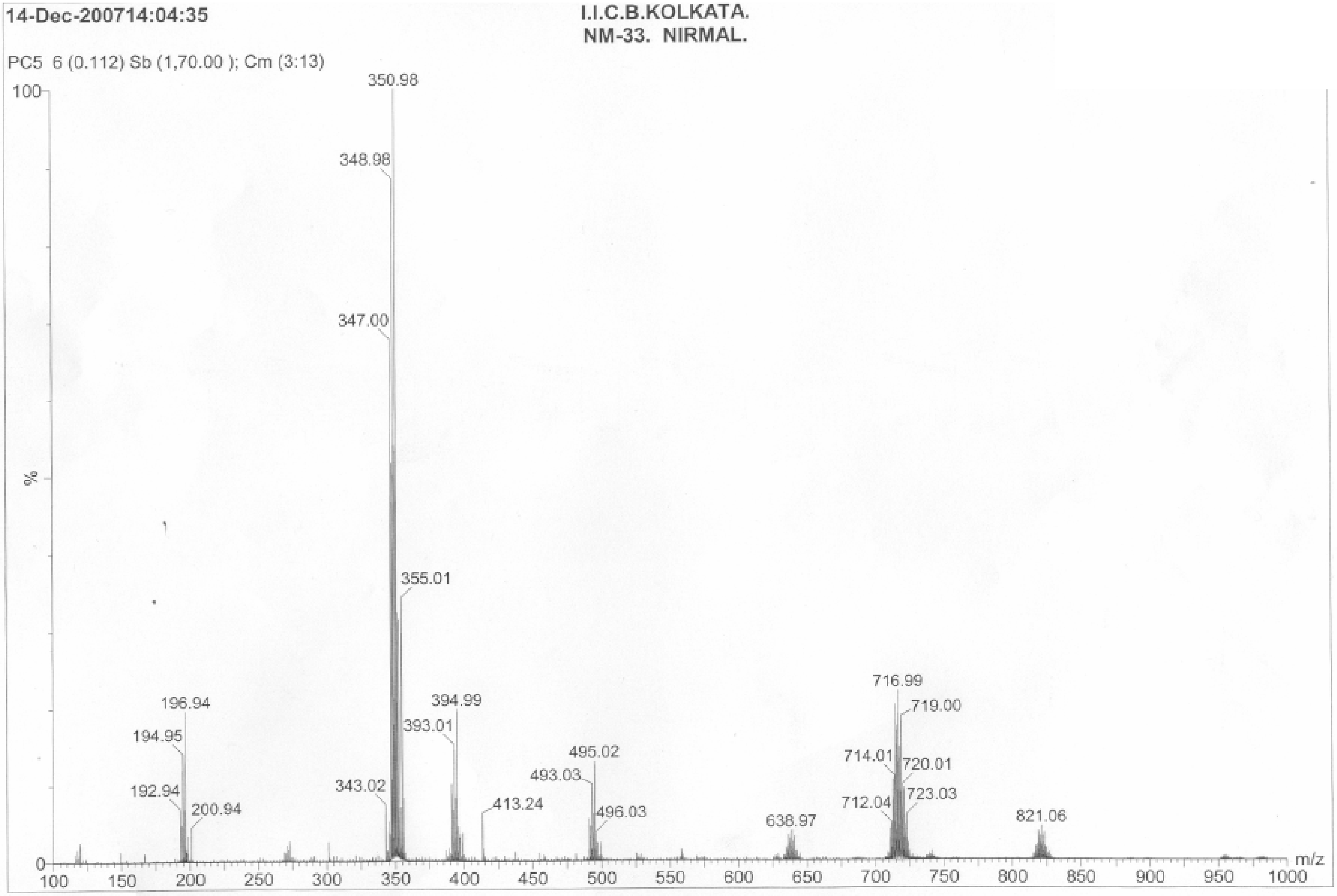


Fig10. Mass spectrum of triphenyltin benzoate

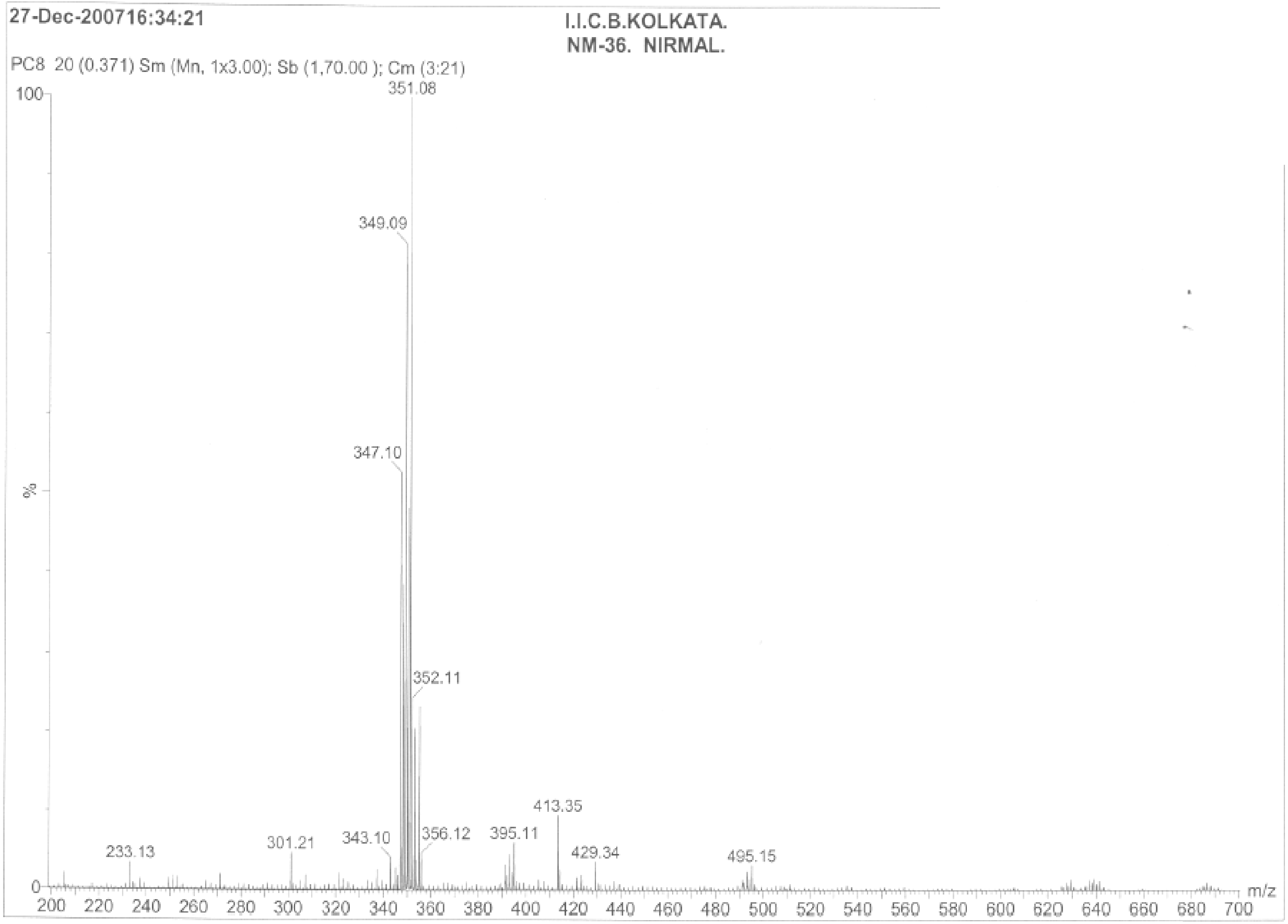


Fig11. Mass spectrum of triphenyltin benzoate aniline