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Synthesis, characterization and biological activities of cephalosporin metals complexes

Rehmat Zaman[1], Wajid Rehman[2], Mukhtiar Hassan[1], Muhammad Mumtaz khan[3] Zeeshan Anjum[5], Syed Asad Hussain Shah[4], Syed Rizwan Abbas[*4]

[1]*Department of Biochemistry, Hazara University, Mansehra, Pakistan*

[2]*Department of Chemistry, Hazara University, Mansehra, Pakistan*

[3]*Department of Microbiology, Haripur University, Haripur, Pakistan*

[4]*Department of Biotechnology, University of Kotli, Kotli, Pakistan.*

[5]*Department of Biotechnology, University of Azad Jammu Kashmir, Muzaffarabad, Pakistan.*

Key words: Cephalosporin, Syntheses, Characterization, Biological activities

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Abstract

Cephalosporin is widely used group prescribed as broad-spectrum antibiotic, containing β-lactam ring. Numerous different bacteriological pathogens are now resistant against cephalosporin due to over use. Subsequently it is necessary to synthesize new derivatives to enhance biological activity against pathogens. In current study cephalosporin were reacted with essential trace elements to synthesize respective metal complexes. The novel compounds were characterized at different spectroscopic techniques like UV, FTIR, HPLC and AAS (atomic absorption spectroscopy). Biological screening of novel compounds was accomplished against diverse group of pathogens. Some of our novel compounds showed high efficacy against the pathogens as compare to parent composites. We have introduced Novel method for ceftriaxone assays at HPLC using simple and safe mobile phase through metal complexation. Toxicity studies were carried out in vitro and with ADMET software. Novel complexes were found less toxic as compare to parent compounds.

* **Corresponding Author:** Rizwan Abbas ✉ drsyedrizwanabbas@gmail.com

Introduction

Cephalosporin antibiotics consist of variety of compounds having different spectrums of activity and pharmacokinetics. All members of this group are derived from cephalosporin C which is obtained from *Cephalosporium acremonium*. Cephalosporins are bactericidal and act by restricting mucopeptide synthesis resulting defective cell wall. Definite mechanism is not fully known but their Beta lactam ring binds with several enzymes like corboxypeptidases, endopeptidases and transpeptidases which are involved in cell wall synthesis (Anacona *et al.*, 2013). Different spectrums of activity are shown due to different affinity of beta lactam antibiotics toward enzymes. Cephalosporin is wide-ranging class of antibiotics conferring to its efficacy against gram positive and gram negative pathogen, grouped in four generations. Group four is broad spectrum antibiotics works thriving against both grams positive and grams negative.

Cephalosporins are abundantly used as broad spectrum antibiotics. According to the WHO china alone consumes more than 1000 ton/anum. Most of the microbes became resistant to cephalosporin due to heavy usage (Jacoby, 2005). Ciprofloxacin has been reported resistant against Escherichia Coli strain in Beijing Hospitals (Wang *et al.*, 2001). *Streptococcu spneumonia* is resistant to most of the Cephalosporins (Davidson *et al.*, 2002). Gonorrhea infection has also been reported resistant to fluoroquinolones. (Newman *et al.*, 2004). *Salmonella, Shigella*, and Campylobacter species has also been reported resistant tocephalosporins. (Alfredson and Korolik, 2007) Keeping this special issue with Cephalosporin we are interested to change the chemistry of this value molecule.

It has been observed that complexation reduces the toxicity of drugs Pathogens are more sensitive to metal complexes as compared to parent compounds (Auda *et al.*, 2008). The molecular diagram indicates cephalosporin possesses– NH_2,- COOH,- CO and N-C and work as ligand. So it form chelatecomplex with essential trace elements like Iron, zinc, cobalt, nickel and chromium (Reiss *et al.*, 2014).

Metal complexes play important role in curing diseases e.g. Cu complexes are effective against, tuberculosis, cancer and rheumatoid arthritis (Williams, 1971).

Cisplatin frequently used in cancer therapy. Similarly Silver and Bismuth complexes show good efficacy against pseudomonas, breast cancer and ulcer respectively (Curran, 2009). Moreover, these ligands bind to essential trace elements in body and may decrease their bioavailability. Deficiency of these essential trace element in body may cause some physiological disorder (Chitterjea, 1993).

Aims of current study were Synthesis, characterization and predicting biological activities of Cephalosporin Metals Complexes. In our study most of essential trace elements like Fe, Zn, Cu, were coordinated with Ceftriaxone, Cefotaxime, Cefoperazone, Cefepimeand Ceftazidime. So intake of theses complexes reduces the risk of deficiency of this essential trace element in the body.

Materials and methods

Chemicals

Metal salts of analytical grade were purchased from Merck. These salts were used without further purification. Cephalosporins were purchased from Harbain Pharmaceuticals china.

Synthesis

Synthesis was carried out in reflux condensation under controlled conditions.

Characterization techniques

UV spectra recorded at single beam Germany IRMICO. HPLC chromatogram recorded at D-star American isocratic system. Melting points were measured at gallon kam apparatus. Shimatzoo 300 was used for Atomic absorption. IR spectra at SEN sir IR spectrophotometer.

Biological screening

Biological activities under LFC having efficiency 99.99 % by disc diffusion method. Toxicological study carried out at albino rats. ADMET (**A**bsorption, Distribution, Metabolism, Excretion and Toxicity profiles) Sar Software was used for toxicity prediction.

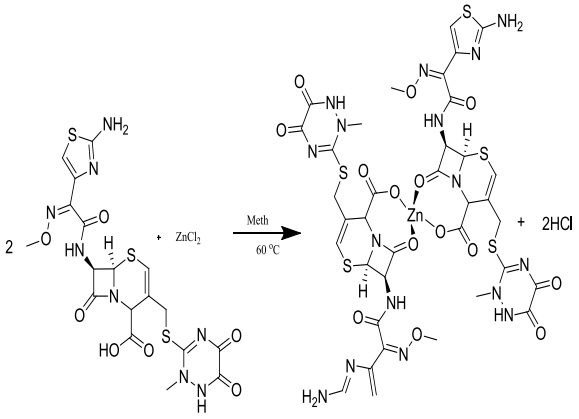
Syntheses of cephalosporin complexes

The cephalosporin-metal complexes were synthesize by mixing the cephalosporin and metal salts in a ratio of 2:1 or 1:1 mmol dissolved in methanol (Auda *et al.*, 2009; Anacona JR, (2006)). The reaction mixture was then refluxed at room temperature for 30 min and then left to stand overnight. The precipitated complexes were filtered off, washed with ether and dried under reduced pressure at room temperature. The synthesis procedures were carried under the normal condition.

The complexes are colored, insoluble in water and other common organic solvents such as ethanol, benzene acetone, acetonitrile, diethyl ether, but they are soluble in dimethyl form amide (DMF) and dimethyl sulfoxide. New compounds were confirmed through various spectroscopic techniques like IR, UV, Atomic absorption and HPLC. The IR spectra were compared with previous studies for similar functional groups and best co relate with results. (El-Said *et al.*, 2009). UV spectra also justified the formation of New complexes (Aly *et al.*, 2004).

Ceftriaxone Zinc complexes

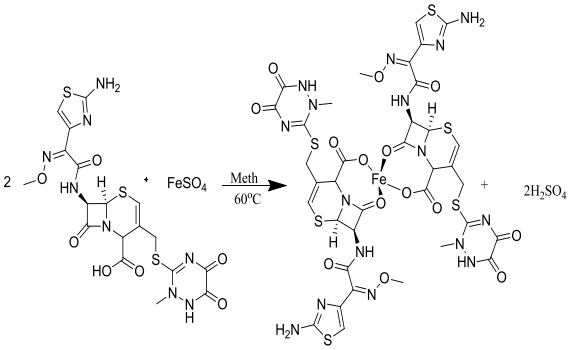
Ceftriaxone (1111.16 mg) reacted with 136 mg Zinc-chloride (2:1) in methanol and reflux for overnight. After filtration precipitate was washed with ethanol and ether (Yield 70%).Complexes were insoluble in organic solvent partially soluble in DMSO. Melting point of ceftriaxone is 236°C while zinc complex is 250°C.



Formation of zinc ceftriaxone.

Ceftriaxone Iron complexes

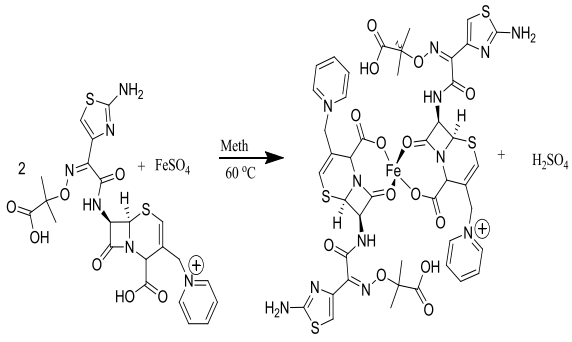
Ceftriaxone (555.58 mg) reacted with 392 mg of Ironsulphate in methanol and reflux for overnight and settled. After filtration precipitate was washed with ethanol and ether (Yield 80%, MP 255°C).



Formation of Iron ceftriaxone.

Ceftazidime Iron complexes

Ceftazidime (1100 mg) pentahydrate reacted with Iron sulphate 800 mg in methanol and reflux overnight (yield 85%, MP of Ceftazidime is 196°C, Complex MP is 260°C).



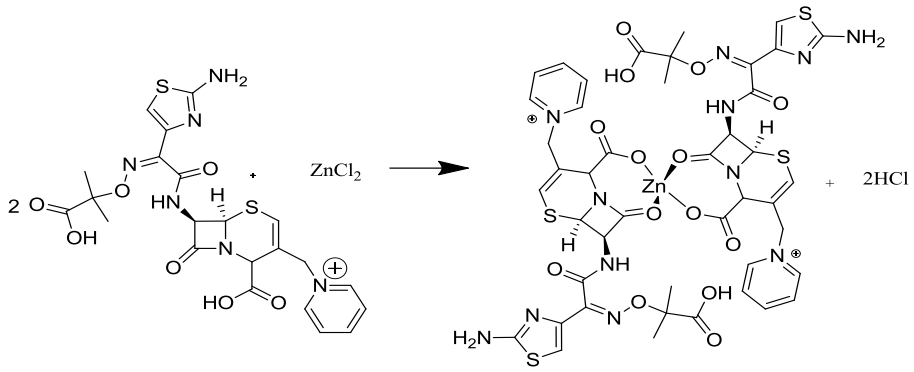
Formation of Iron Ceftazidime.

Ceftazidime Zinc complexes

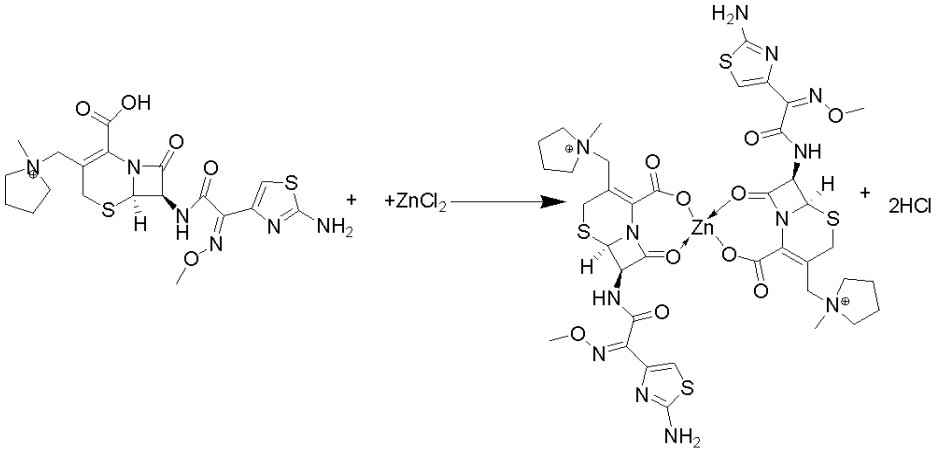
440 mg of Ceftazidime reacted with 320 mg of Zinc chloride (2:1) in methanol reflux overnight. Initially the color of solution was blue and then turn brick red (yields 90%, MP 255°C).

Cefepime Zinc complexes

Cefepime (960mg), arginine (392 mg) and zinc chloride (272mg) reacted in methanol and reflux overnight resulting white complex. After filtration precipitate was washed with ether after drying it become orange compact crystals (yields 75%).



Formation of zinc ceftazidime.



Zinc ceftazidime.

Results and Discussion

Infra-Red spectrophotometry (IR)

Novel metal complexes were confirmed from the IR spectra which is different from its parent compound. IR spectra were recorded on SENsir IR spectrophotometer in the range of 4000cm-1 –1000 cm-1.IR spectral analyses of Zn ceftriaxone complex show that stretching frequency vibrations at1700 cm-1 of the β–lactam carbonyl group corresponding to ceftriaxone has been shifted to higher frequency 1800 cm-1 this shows the involvement of this group in metal complex formation.

Further the asymmetric and symmetric stretching vibrations of carboxylates appear at 1530-1400 and 1400-1410 in free ligand and Zinc complex respectively. This also shows involvement of this group in coordinate complex. The Stretching frequencies vibrations corresponding to amide carbonyl group appear without significant change i.e. C=O for amid at 1600 cm-1 appear both in parent and ceftriaxone zinc complex. This shows that group does not take part in complex formation Table 1, Fig. 1-2. Thus ceftriaxone act as bi dentate ligand in zinc metal complex.

Table 1. Main vibrational frequencies, υ (cm–¹) of Cef metal complexes.

Compound	υ (C=O Lact)	υ (C=O amide)	υ(COO) Asym	υ(COO)sym	Δυ
Ceftriaxone	1700	1600	1530	1400	130
Ceftriaxone Zinc	1800	1600	1400	1410	10
Cefepime HCl	1850	1600	1520	1320	200
Cefepime zinc	1970	1600	1400	1050	350
Ceftazidime5H2 O	1790	1600	1550	1225	325
Ceftazidime Zn	1730	1590	1400	1190	220

(IR spectral data for cephalosporin and their metal complexes.)

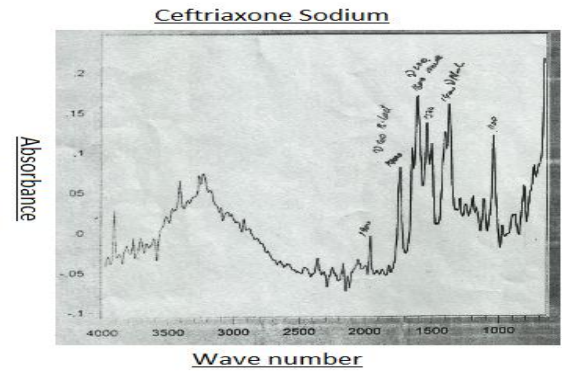


Fig. 1. IR spectra for ceftriaxone.

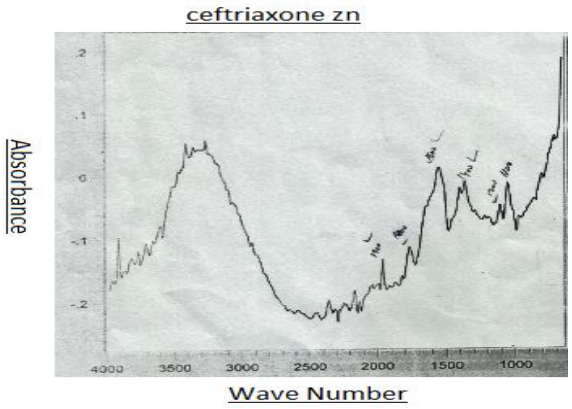


Fig. 2. IR spectra for ceftriaxone Zinc.

Similarly stretching frequency vibrations of the β–lactam carbonyl group corresponding for Cefepime HCl 1850cm^{-1} has been shifted to higher frequency 1970cm^{-1}in Cefepime zinc complexes. The asymmetric and symmetric stretching vibration of carboxylates bands at 1520-1320 for has been shifted to 1400-1050 respectively in Zinc complexes. This reveals that both carbonyl and carboxylate groups are involved in coordination Fig. 3-4.

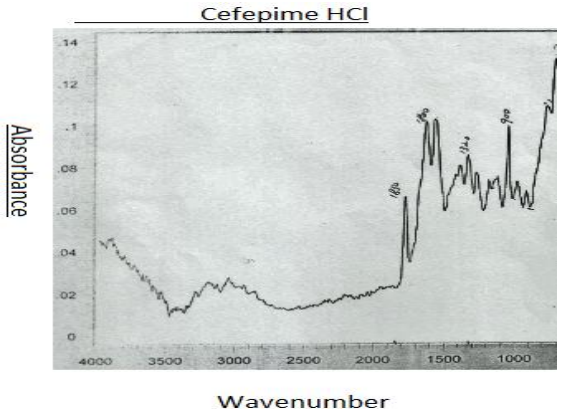


Fig. 3. IR spectra for cefepime HCL.

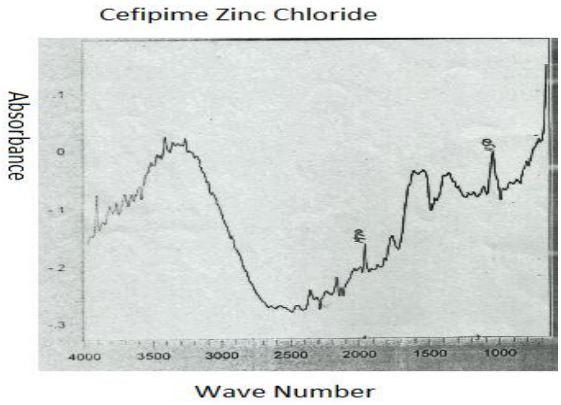


Fig. 4. IR spectra for cefepime zinc choliride.

Surprisingly same mode of interaction appeared in ceftazidime i.e. stretching frequency vibration sat 1790cm^{-1} of the β–lactam carbonyl group corresponding to ceftazidime molecule has been shifted to lower frequency 1730cm^{-1}. The asymmetric and symmetric stretching vibrations of carboxylates appear at 1550-1225 and 1400-1190 cm^{-1}in free ligand and Zinc complex respectively Fig. 5-6.

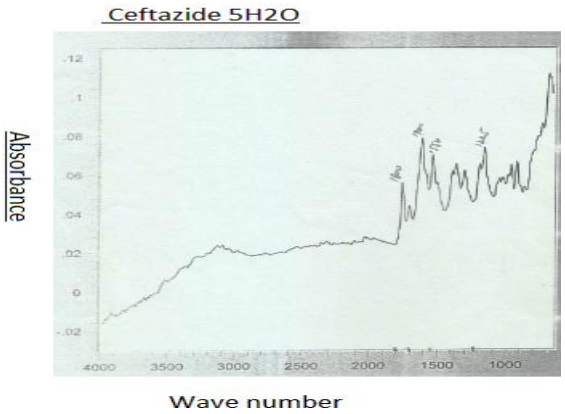


Fig. 5. IR Spectra for Ceftazidime.

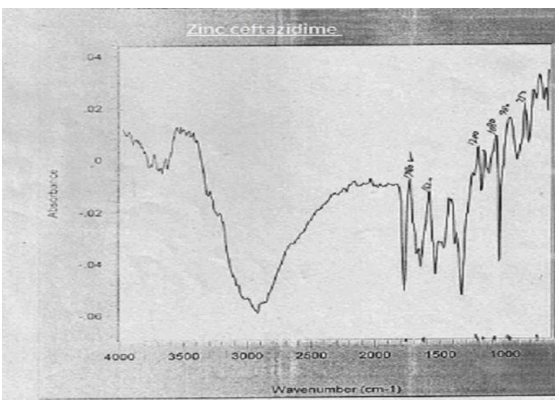


Fig. 6. IR spectrafor zinc ceftazidime cholride.

Ultra violet and visible spectroscopic study

UV spectra were recorded between 200 nm to 400 nm. UV scan of Zinc complexes in DMF show no change in Lambda max indicates that there are no d-d transitions of electron occur when legend binds to the zinc metal, Fig. 7-8. So due to complete filled d orbital d^{10} of zinc it is not possible to determine the bonding with UV spectrophotometer. Ale *et al also* observe similar spectra in his thermal and photo chemical study of some zinc complexes.

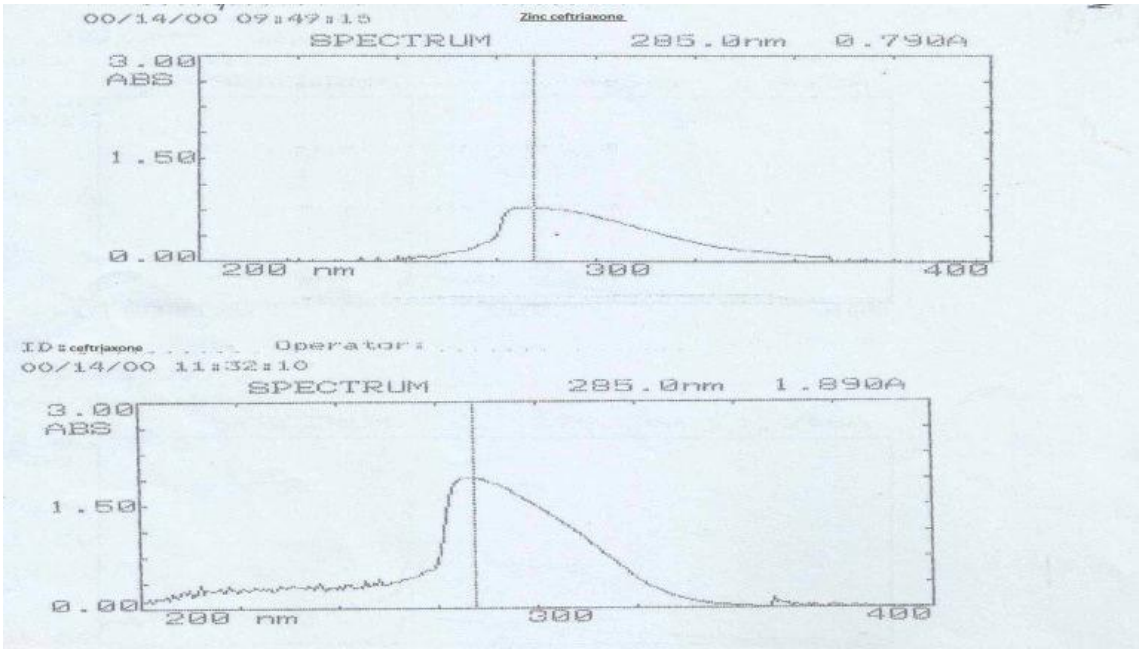


Fig. 7. UV spectra for ceftriaxone and zinc ceftriaxone chloride.

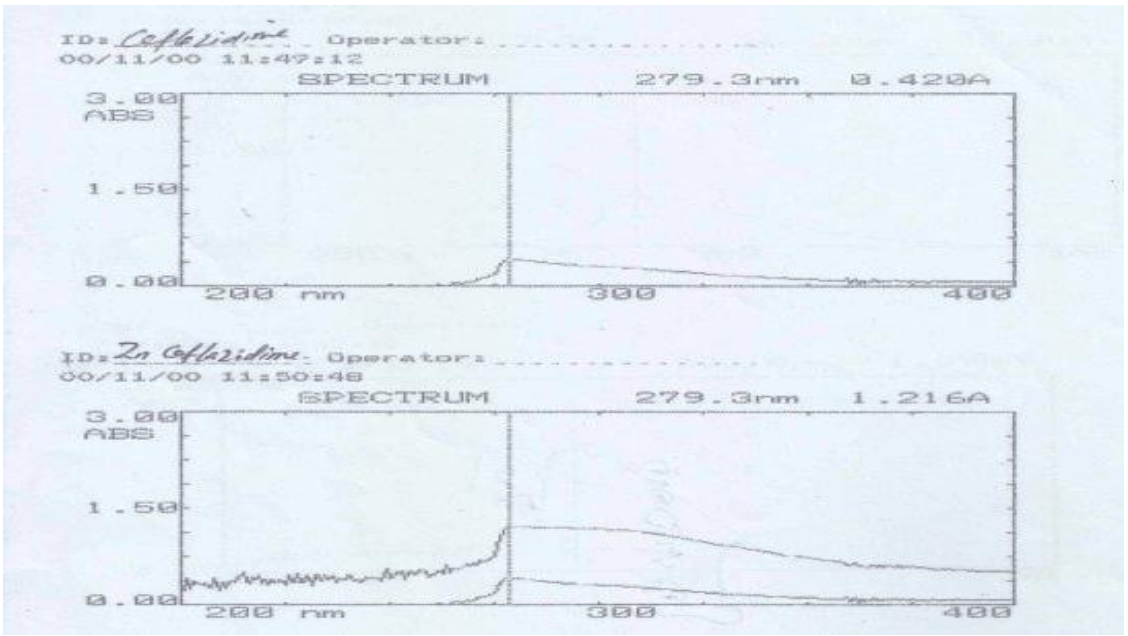


Fig. 8. UV Spectra for ceftazidime and Zinc ceftazidime chloride.

Atomic absorption analyses

The quantitative analyses were carried out by atomic absorption metal analyses shimatzoo 300. The experimental results were compared with theoretical results which shows each metal calculated experimentally comply with theoretical results Table 2.

Table 2. Zone of inhibition in mm (Disc containing 1 mg in water/disc).

Compounds	*Klebsella Pneumonia*	*Staphylococcus Strains*	*E. coli*	*Acinetobacter*
Ceftriaxone Na	24.8	9.29	18.97	7.33
Ceftriaxone Fe.	20.77	15.07	7.02	10.5
Ceftriaxone Zn	5.0	8.12	12	9.7
Ceftriaxone Cu	10	**10**	13	10.0
Ceftazidime	17.88.12	10.78	15.09	13.35
Ceftazidim Zn	15	12.32	12	14
Ceftazidim Fe	16	13.45	13.4	13.3
Ceftazidim Cu	12	12.54	16.3	12.42
Cefepime	21.97	15.98	20.44	22.51
Cefepime Zn	22	16.32	15	23

(Antibacterial activity of cephalosporin and metal complexes).

High performance liquid chromatography

Previously no HPLC work is reported for cephalosporin complex. The mobile phase was prepared by using HPLC grade acetonitrile and distilled water (500ml + 500ml). This was filter and degassed. We obtain chromatogram at flow of 1ml/min using column C18 at wavelength 230. We did not adopt the USP (United States pharmacopeia) method rather we developed new mobile phase for this complex. This new protocol is good for column as it has no buffering salt which may be harmful for column if washed improperly. The chromatogram of metal complexes indicated somewhat lower retention time than reference ceftriaxone, it may be due to high molecular weight of complexes. The retention time for ceftriaxone started at 1.07 and peak ended at 2.15 mints while retention time for metals started exactly at 1.00 mints and ended at 1.61 mints. Thus the time span for the metal complex was less than the original drug and gave much sharp peak. Further metal complexes showed sharp peak and there was no shoulder as in ceftriaxone reference raw material Fig. 10-9. This sharp peak support in validation in assays of ceftriaxone drug during its manufacturing and give more accuracy and precession.

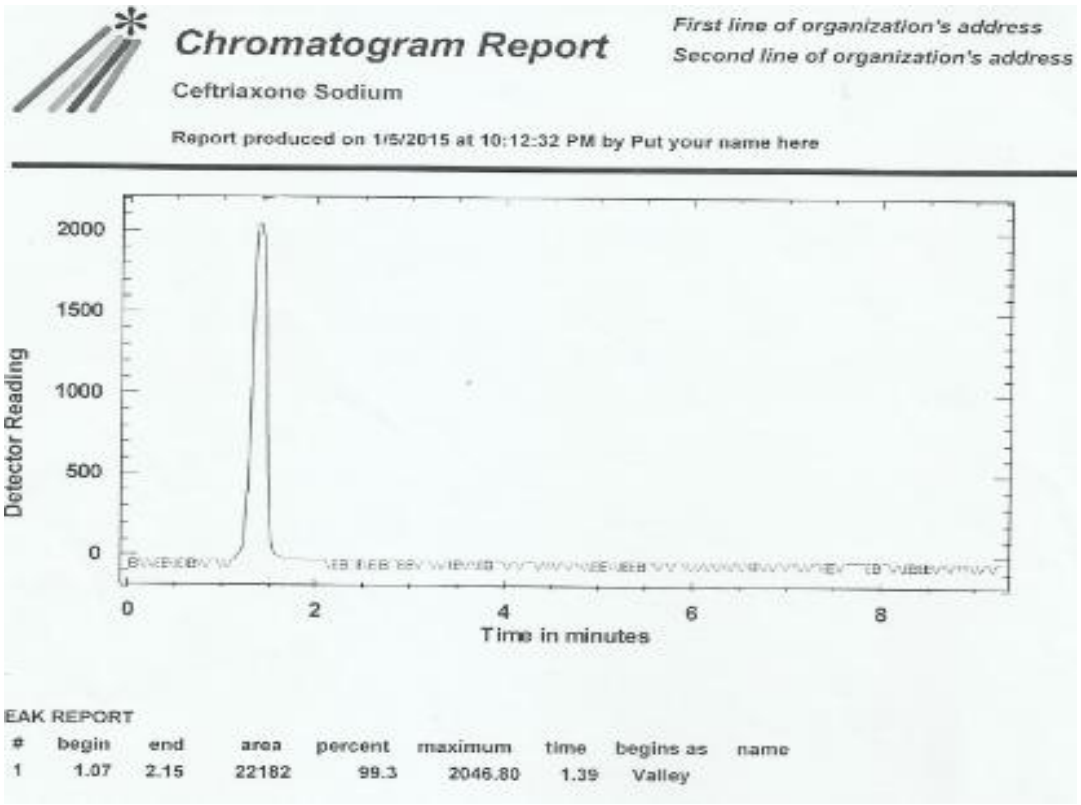


Fig. 9. HPLC chromatogram for ceftriaxone sodium.

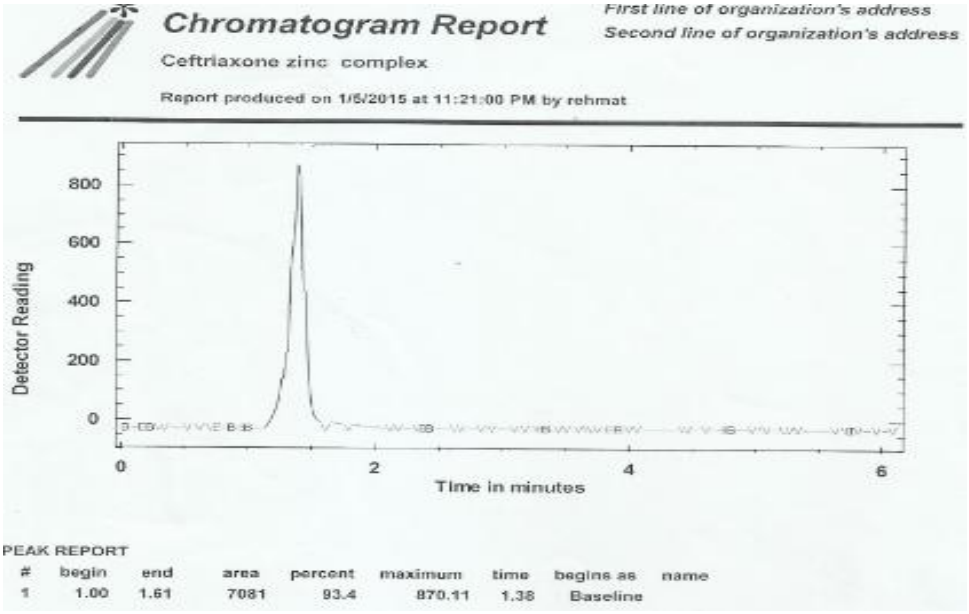


Fig. 10. HPLC chromatogram for Zinc ceftriaxone complex showing sharp peak and low retention time for zinc complex.

Toxicity study

It has been observed that metal complication lowers the toxicity of drugs. The toxicity study carried out at albino rates. To check lethal dose LD_{50}, Dose increases from 50mg/kg body weight administered in vein. After administration rates keep under observation, Table 3 results showed that metal complexes are less toxic as compare to the parent cephalosporin. Toxicity through ADMET profile also shows that metal complexes are less toxic then the parent compounds. It is conform from the table that Ceftriaxone Zn with Lethal dose 11 GM/KG experimental and 2.4979mol/kg calculated is less toxic then ceftriaxone with Lethal dose 5 GM/KG experimental and 2.4825mol/kg calculated. Ceftazidime-Zn with Lethal dose 9 GM/KG experimental and 2.2786mol/kg calculated is less toxic then Ceftazidime with Lethal dose 6GM/KG experimental and 1.8229mol/kg calculated. Similarly Cefepime Zn with Lethal dose 8 GM/KG experimental and 2.5300mol/kg calculated is less toxic then Cefepime with Lethal dose 5 GM/KG experimental and 2.3058mol/kg calculated through ADMET software, Table 3.

Table 3. LD_{50} (quantity resulting the death of half of the rats and ADMET Values).

Compound name	Normal dose MG/KG	Lethal dose GM/KG	ADMET mol/kg
Ceftriaxone Na	50	5	2.4825
Ceftriaxone Zn	50	11	2.4979
Ceftazidime 5H20	50	6	1.8229
Ceftazidime Zn	50	9	2.2786
Cefepime HCl	50	5	2.3058
Cefepime Zn	50	8	2.5300

(Toxicity evaluation of cephalosporin and metal complexes).

Minimum inhibitory concentration (MIC)

The zone of inhibition was calculated using digital Vernier caliper by disc diffusion methods. Table 4 shows that some of metal complexes show greater efficacy then the parent cephalosporin. Fewer efficacies may be due to the low solubility of metal complexes in water.

Fig. 11 shows that Iron-ceftriaxone complexes show greater activities against *Staphylococcus* while other *metal* complexes show equal are fewer efficacies. Table 4 Fig .12 also shows that E. *Coli* are more sensitive toward cu ceftriaxone complexes. It has been observed that *Acinobacter* are more sensitive toward iron ceftriaxone and cu ceftriaxone as compare to parent ceftriaxone, Fig. 13.

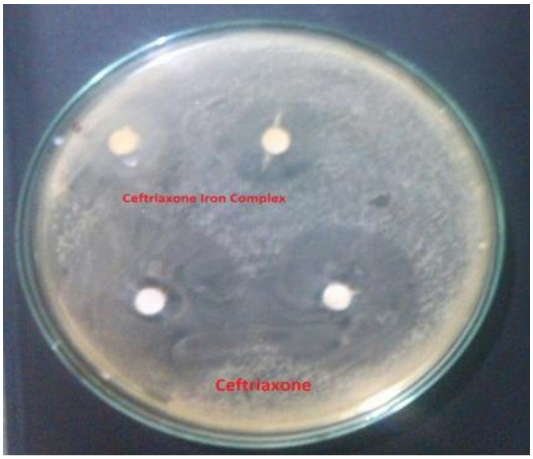


Fig. 11. Staphylococcus sensitivity and resistance pattern against ceftriaxone iron and ceftriaxone drug.

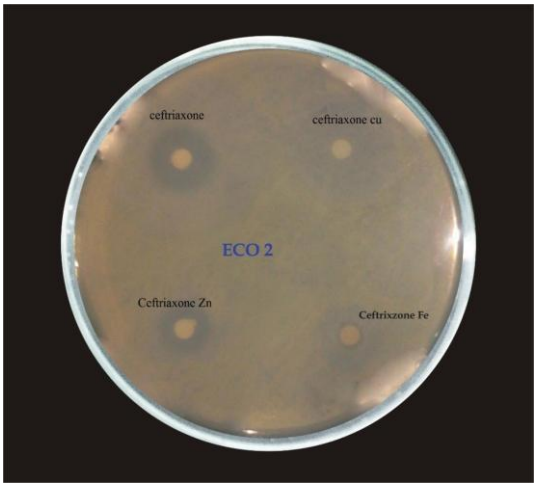


Fig. 12. Zone of inhibition of ceftriaxone and its metal complexes against E coli.

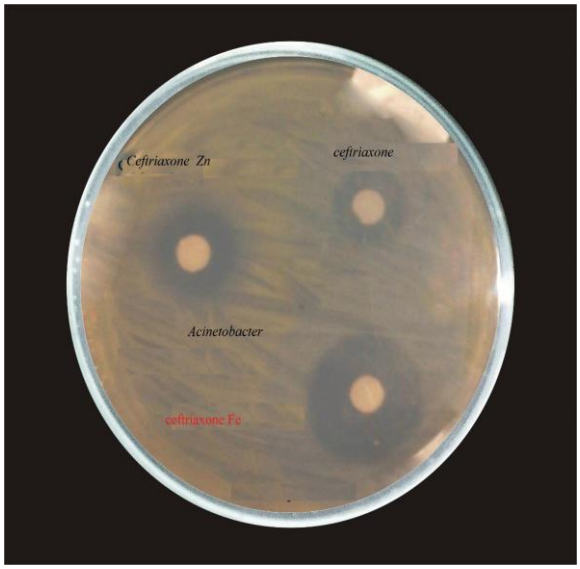


Fig. 13. Zone of inhibition of ceftriaxone and its metal complexes acinobacter**.**

Iron, Zinc and Coper complexes with ceftriaxone shows greater activities against *klebsela pneumonias*. Zinc and Iron complexes with ceftazidime shows greater activities against *klebsela pneumonias* while Its copper complexes show less efficacy.

It may be due less solubility in the solvent. Zinc-cefepime also shows high efficacy against *klebsela pneumonias* Table 4.

Table 4. Atomic absorption.

Compounds	Molecular weight	Theoretical % age of metal	Experimental results in ppm	Experimental %age of metal(20mg/ 50 ml)
Zn (ceftriaxone)	620.19	10.5 %	37	9.25%
Fe (Ceftriaxone)	610.6	10.06	39	9.75 %
Zn (Cefepime)	545.4	11.99	41	10.25
Fe (Cefepime)	535.8	10.4	36	9.00
Zn (Ceftazidime)	611.99	10.68	38	9.5
Fe (Ceftazidime	602.38	10.1	39	9.75

Conclusions

Zinc and Iron is an essential trace element in the body. Cephalosporin chelates these essential trace elements in ratio of 2:1Zinc and Iron respectively. Cephalosporin overuse may cause deficiency of minerals and serious physiological disorders. Pathogens became resistant toward parent compounds

So modification in existent molecules is required. Our novel compounds are highly efficient toward tested pathogens.

Staphylococcus and *Acinetobacter*s showed more sensitivity against the novel Iron-ceftriaxone, Zinc–ceftriaxone, Iron-Ceftazi S Sdime, Zinc-Ceftazidimeand Zinc-Cefepime complexes. Metal complexes were found less toxic then the parent compounds.

HPLC results of metals complexes are useful in chemical analyses of the drugs as it gave sharp peak. Thus we have established new safe and rapid protocol at HPLC for Ceftriaxone drug assay that can be validated.

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