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SYNTHESIS AND PHYSICOCHEMICAL CHARACTERIZATION OF CEFDINIR PRODRUGS

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ABSTRACT

Cefdinir is one of the third generation cephalosporin which has broad spectrum antibacterial activity. Cefdinir has activity against Gram-positive and Gram-negative bacteria. It is also effective against β-lactamase producing strains of haemophilis and neisseria. This drug is used to treat wide variety of sensitive bacterial infections, especially for mild and moderate infections disorders. The success of cefdinir is limited due to poor permeability of drug across cell membrane. Prodrug is one of the strategies to reduce the required dose of the drugs in order to achieve the desired bioavailability with enhanced permeability. In the present study, three different prodrugs of cefdinir (cefdinir methyl ester (CEF-1), cefdinir ethyl ester (CEF-2) and cefdinir benzyl ester (CEF-3) were synthesized. Further they were evaluated for physicochemical properties including solubility and partition coefficient. Ester prodrugs were found to be more soluble at pH 1.2 whereas cefdinir was found to have solubility at pH 1.2 and pH 7.4 due to amphoteric nature. Both drug as well as prodrugs was found to be stable at pH 1.2 as compared to pH 7.4. Additionally introduction of ester group in cefdinir increased the lipophilicity as observed in partition coefficient study. Prodrugs were found to be more lipophilic than cefdinir and were found to be interesting for further *in-vivo* animal study.

KEYWORD: lipophilicity, haemophilis and Neisseria.

INTRODUCTION

The potency, safety and financial investment of chemical entities are important issues for development of new drug molecules. The therapeutic efficacy can be improved by overcoming the undesirable properties while retaining the desirable ones. This can be achieved through biological, physical or chemical ways. The biological approach is to alter the route of administration which may or may not be acceptable to patient. The physical approach is to modify the design of dosage form such as controlled drug delivery of drug. The third and the best approach in enhancing drug selectivity while minimizing toxicity, is the chemical approach for designing prodrugs.[1]

The main rationale underlying synthesis of prodrug esters is to enhance lipophilicity, and thus the passive membrane permeability of poorly permeable drugs. To increase the lipophilicity of carboxyl group bearing pharmacologically active compounds, they can be esterified with aliphatic or aromatic alcohols.[2-4]

Cefdinir is one of the third generation cephalosporin which have broad spectrum antibacterial activity.[5] Cefdinir has activity against gram-positive and gram-negative bacteria. It is also effective against β-lactamase producing strains of haemophilis and neisseria. This drug is used to treat wide variety of sensitive bacterial infections, especially for mild and moderate infections.[6] Cefdinir is a BCS class IV drug having a limited bioavailability due to poor permeation.[7] So in the present study an attempt has been made to prepare different prodrugs of cefdinir and characterize them substantially including physicochemical parameters and their structure elucidation.

MATERIALS AND METHODS

Cefdinir was obtained from Saga Laboratory, Ahmedabad. Cesium carbonate and 1-octanol was purchased from Sigma Aldrich. All other solvents used were of analytical grade obtained from finar chemicals.

Synthesis of prodrugs of Cefdinir

The synthesis of prodrugs was carried out in the following way:

Synthesis of Cefdinir methyl ester (CEF-1)

Cefdinir (1.18 grams, 3 mmol) and cesium carbonate (1.46 grams, 3 milli eq.) were added to round bottom flask containing dimethyl formamide (10ml) and was stirred till clear solution

was obtained. The reaction mixture obtained above was stirred further on ice bath at temperature 2-4 ^{o} C for 15 minutes and followed by addition of methyl iodide (0.51 grams, 3 milli eq.). The reaction mixture was stirred for about 6 hrs. at room temperature till precipitate formation is ceased. The reaction mixture was filtered to yield orange colored precipitates (Yield, 68%). Precipitates obtained were washed alternatively with saturated sodium bicarbonate solution and distilled water and kept for drying.[8,9]

Synthesis of Cefdinir Ethyl ester (CEF-2)

Cefdinir (1.18 grams, 3 mmol) and cesium carbonate (1.46 grams, 3 milli eq.) were added to round bottom flask containing dimethyl formamide (10ml) and was stirred till clear solution was obtained. The reaction mixture obtained above was stirred further on ice bath at temperature 2-4 ^{o}C for 15 minutes and followed by addition of ethyl bromide (0.27 ml, 3 milli eq.). The reaction mixture was stirred for about 4 hrs at room temperature till precipitate formation is ceased. The reaction mixture was filtered to brown colored precipitates (Yield 59%). Precipitates obtained were washed alternatively with saturated sodium bicarbonate solution and distilled water and kept for drying.[10,11]

Synthesis of Cefdinir Benzyl ester (CEF-3)

Cefdinir (1.18 grams, 3 mmol) and cesium carbonate (1.46 grams, 3 milli eq.) were added to round bottom flask containing dimethyl formamide (10ml) and was stirred till clear solution was obtained. The reaction mixture obtained above was stirred further on ice bath at temperature 2-4C for 15 minutes and followed by addition of benzyl chloride (0.42 ml, 3 milli eq.). The reaction mixture was stirred for about 6 hrs at room temperature till precipitate formation is ceased. The reaction mixture was filtered to dark orange colored precipitates (Yield 72%). Precipitates obtained were washed alternatively with saturated sodium bicarbonate solution and distilled water and kept for drying.[12,13]

Fig 1 shows the synthetic scheme used to synthesize CEF-1, CEF-2, CEF-3.

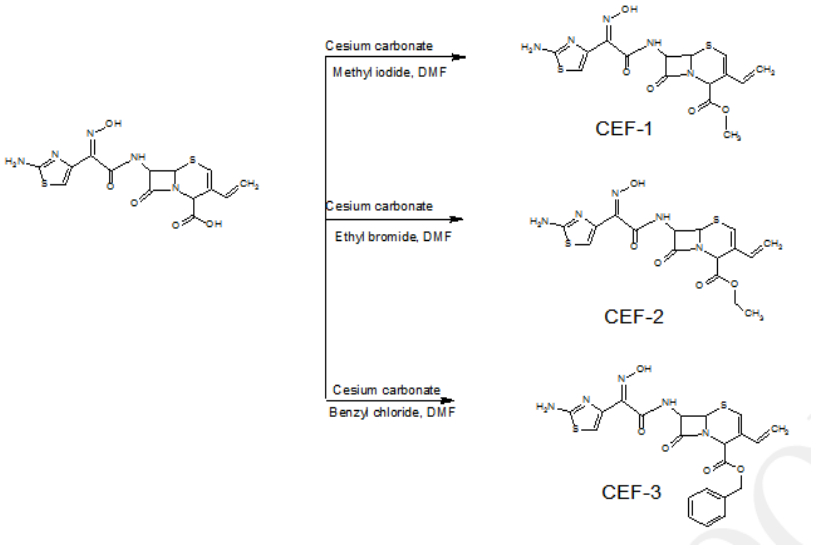

Figure: 1. Scheme for synthesizing CEF-1, CEF-2 and CEF-3.

Analysis of prodrugs[14]

Waters Alliance system with UV detector and analytical column Kromasil ^{100} RP-18(250 x4.6mm, 5µm) from Merck was used for Cefdinir and its pro-drugs' quantification. Cefdinir and its pro-drugs' were separated and eluted isocratically at a flow rate of 1 ml/min using mobile phase concentration of Methanol:0.2M phosphate buffer pH 3.0 (30:70). Injection volume was 20 µl and retention time of Cefdinir was 3 min. Pro-drugs Cef-1, Cef-2 and Cef-3 were eluted at 5.6, 7.8 and 12.2 minute respectively. Peaks were measured at a wavelength of 254 nm.

Physicochemical properties

Solubility measurements

A solubility study of Cefdinir and its prodrugs (CEF-1, CEF-2, and CEF-3) was carried out by the method of Okumara et al.[15] An excess amount of drug and prodrugs[16] were added and dissolved in 10ml of buffer (pH = 1.2, 4.5, 6.8 and 7.4) in a glass vial to get a saturated solution. The system was stirred for 24 h at 37˚C and kept at rest for 1 h to assist the attainment of equilibrium. The solution was then filtered through a membrane filter (pore size 0.22 µm) and after dilution, the solubility of each drug and prodrugs were determined by HPLC.[17,18]

Solution state stability study

The hydrolysis of the synthesized cefdinir and prodrugs was studied in 0.1N hydrochloride acid buffer (pH 1.2) and phosphate buffers (pH 7.4). The constant ionic strength (µ) of 0.5

was maintained for both buffers by adding a calculated amount of potassium chloride.[9] The stock solutions (1 mg/ml) of Cefdinir, CEF-1, CEF-2, and CEf-3 were prepared in DMSO (dimethyl sulfoxide) and dissolved in 10 ml of buffer solutions of different pH 1.2 and 7.4. It was shaken at 37^{o} C and rates of hydrolysis were determined by using HPLC method [19]. The hydrolysis studies were carried out for 24 hours. Samples were taken in regular intervals and drug content was analyzed using HPLC.

Measurement of partition coefficient

The partition coefficient of drug and prodrugs were determined in mutually saturated (at 250˚C for 24 h) n-octanol–pH 7.4 buffer (μ = 0.5) system.[20,21] The partition coefficient study was performed using mixture of 450 µl of n-octanol and 450 µl of phosphate buffer pH 7.4. This mixture of was shaken in thermomixer at 37^{o} C for 24 hours to achieve equilibrium. The 100 µl of drug and prodrug solution (1 mg/ml in DMSO) was added then again it was shaken for 4 hours in thermomixer and centrifuged to separate the layers. Both the layers were injected to HPLC and drug content was calculated. The partition coefficient was determined from the equation.[22]

$$\text{Partition coefficient} = \text{Conc. in octanol layer}/\text{Conc. in buffer layer}$$

Structural characterization

The synthesized cefdinir prodrugs were characterized by FTIR, ^{1}H-NMR and Mass to confirm its structures.

FTIR spectroscopy

The FTIR spectra of Cefdinir and prodrugs were recorded by FTIR instrument (Perkin Elmer G-FTIR, Waltham, MA). Samples in the dried form were crushed and mixed with KBr in the ratio of approximately 1:3. The IR spectra were done against the KBr background. Spectral scanning was done in the range between 4000 and 400cm^{-1}.[23]

NMR spectroscopy

The samples were dissolved in DMSO or $CDCl_3$ to make 5% solutions. The 5% sample solutions were transferred to 5mm NMR tube and the proton NMR spectra were recorded using Bruker Advance II (400 MHz) NMR Spectrometer (Bruker BioSpin AG, Fllanden, Switzerland).[24]

Mass spectroscopy

Mass spectrometry measures the mass of molecules by measuring the mass-to-charge ratio (m/z). Molecular mass analysis was performed by Electron impact (EI) ionization technique and MALDI-TOF.[24]

RESULT AND DISCUSSION

Analysis of prodrugs

The quantification of cefdinir prodrugs by RP HPLC method significantly separated the peaks of Cefdinir and three prodrugs. This infers that synthesized prodrugs are different. **Table 1** indicates the retention time of different prodrugs.

Table.1: Retention time of Cefdinir and cefdinir prodrugs

Prodrug	Retention time
Cefdinir	3.0 min
CEF-1	5.6 min
CEF-2	7.8 min
CEF-3	12.2 min

Physicochemical properties

Solubility study of cefdinir

Solubility of cefdinir and its prodrugs CEF-1, CEF-2 and CEF-3 were performed using buffers of pH 1.2, pH 4.5, pH 6.8 and 7.4. **Table 2** shows that cefdinir is comparably soluble in acidic as well as at neutral pH due to its amphoteric nature. Whereas, prodrugs are more soluble in acidic pH as compared to neutral and basic pH. This may be due to the presence of only ionisable amino group in the prodrugs.

Table: 2. Solubility study of Cefdinir and prodrugs

Drug	Solubility (mg/ml) at given pH			
	1.2	4.5	6.8	7.4
Cefdinir	2.56 ± 0.14	1.90 ± 0.07	1.60 ± 0.06	1.67 ± 0.06
Cef-1	3.52 ± 0.24	2.30 ± 0.23	0.63 ± 0.04	0.53 ± 0.03
Cef-2	3.54 ± 0.27	2.01 ± 0.08	0.69 ± 0.04	0.61 ± 0.02
Cef-3	3.42 ± 0.28	2.15 ± 0.06	0.46 ± 0.05	0.51 ± 0.04

Stability study of cefdinir and its prodrugs

The stability of the prodrug in different pH 1.2 and pH 7.4 is carried out to determine the stability of drug or prodrug before absorption. **Fig. 2 and Fig. 3** show that cefdinir, CEF-1, CEF-2 and CEF-3 are more stable in the acidic media. All prodrugs were found to be stable enough such that they can be absorbed in their intact form. Cefdinir and prodrugs were prone

to hydrolyzed faster at pH 7.4 than pH 1.2. However, hydrolysis of all prodrugs was found to follow first order kinetics.

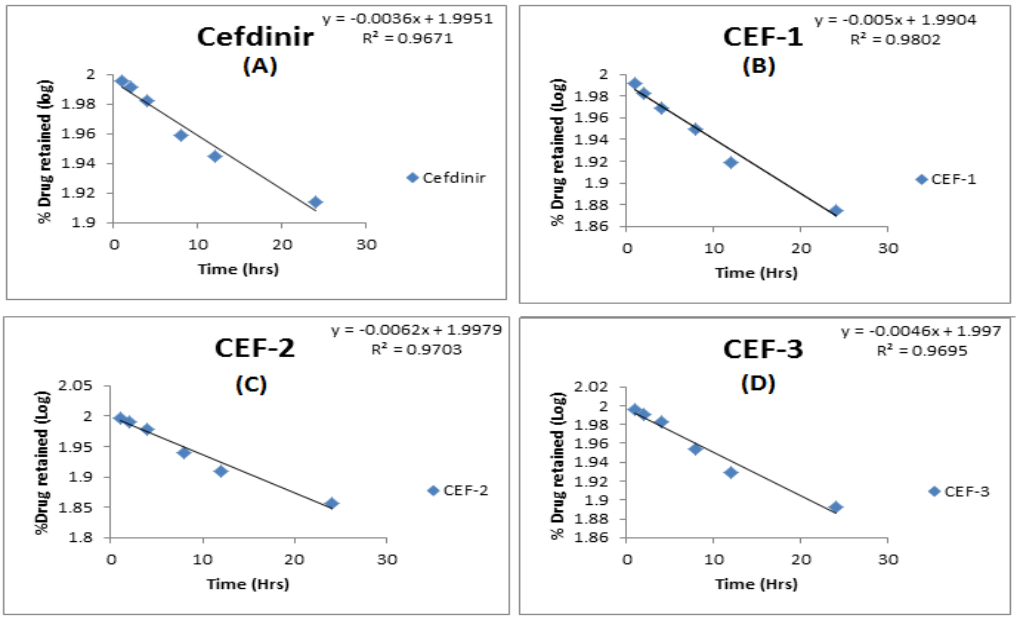

Figure: 2. Solution state stability at pH 1.2 of (A) Cefdinir (B) CEF-1 (C) CEF-2 (D) CEF-3.

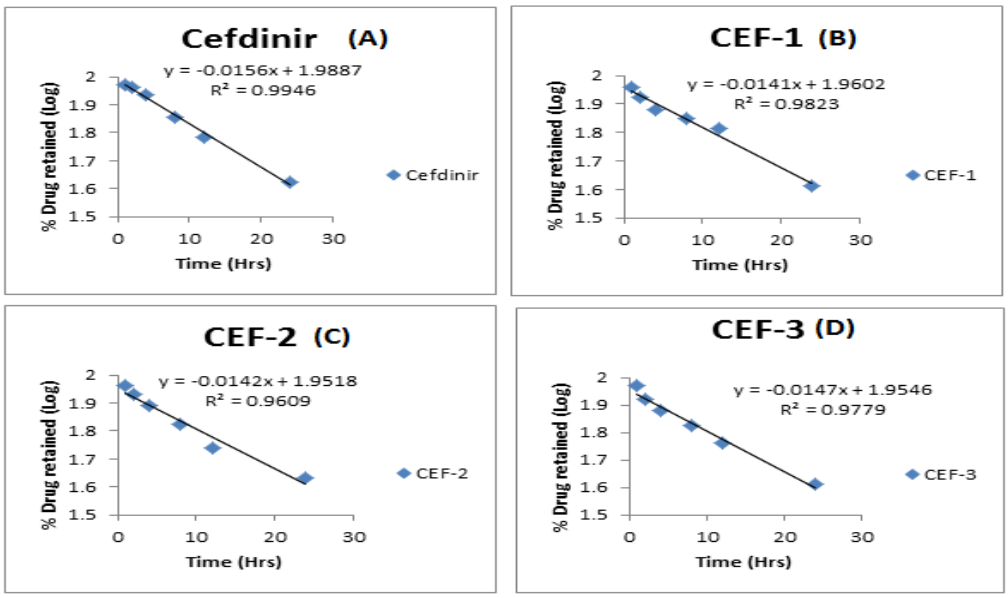

Figure: 3. Solution state stability at pH 7.4 of (A) Cefdinir (B) CEF-1 (C) CEF-2 (D) CEF-3

Determination of partition coefficient of Cefdinir and its prodrugs

Partition coefficient study mainly highlights the change in the order of lipophilicity. **Table 3** shows the LogP values of cefdinir and its prodrugs which clearly indicate that all cefdinir

prodrugs CEF-1, CEF-2 and CEF-3 are more lipophilic than cefdinir. Since it is well known that cefdinir being more polar and amphoteric is less permeable to intestinal wall. All three prodrugs with enhanced lipophilicity may subsequently have higher permeability profile than cefdinir.

Table: 3. Partition co efficient of Cefdinir and its prodrugs

Drug/ Prodrug	LogP at buffer pH 7.4
Cefdinir	-0.89 ± 0.07
Cef-1	0.64 ± 0.06
Cef-2	0.62 ± 0.08
Cef-3	0.78 ± 0.06

STRUCTURAL CHARACTERIZATION

FTIR spectroscopy

The characteristics peak of the CEF-1 and CEF-2 at 1735 cm^{-1} is due to -COOR stretch clearly indicates the presence of ester group. The characteristics peak of the CEF-3 at 1738 cm^{-1} is due to -COOR stretch clearly indicates the presence of ester group. The respective FTIR spectra of CEF-1, CEF-2 and CEF-3 are presented in **Fig. 4, 5** and **6** respectively.

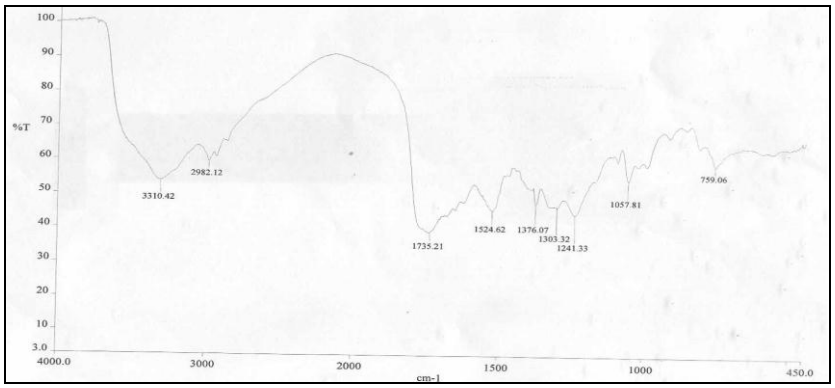

Figure: 4 FTIR spectrum of CEF-1

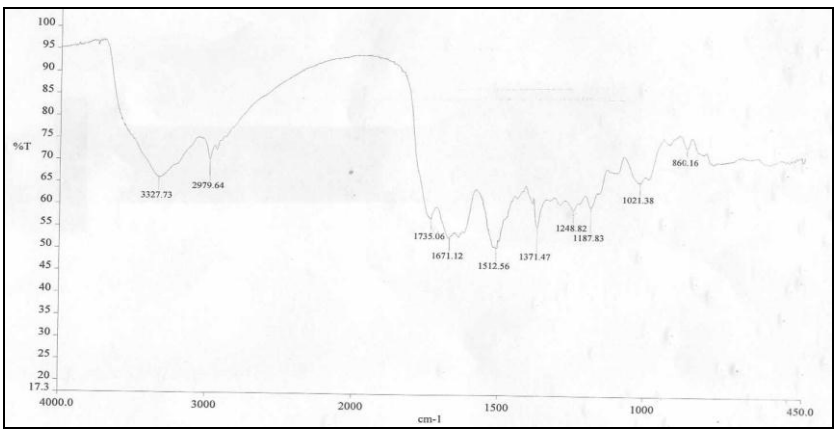

Figure: 5 FTIR spectrum of CEF-2

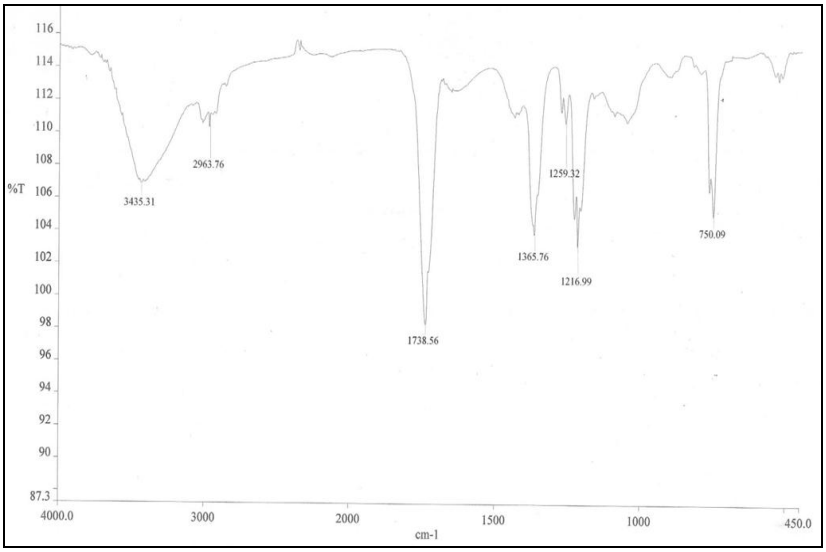

Figure: 6 FTIR spectrum of CEF-3

NMR spectroscopy

CEF-1 ^{1}H NMR (DMSO)

3.48 (bs, 2H (-SCH2)); 3.17 (s, 3H (-OCH3)); 5.16-5.17(d, 1H (C=CH2 ,J= 4.84)); 5.26-5.29 (d, 1H (C=CH2 J=11.4)); 5.52-5.56 (d, 1H (-CH-)); 5.75-5.78 (m, 1H (-CH-)); 6.66 (s, 1H (aromatic –CH); 6.88-6.93 (m, 1H(-CH-)); 7.08-7.12 (d, 4H (-NH-, -OH, -NH2)).

CEF-2^{1}H NMR ($CDCl_3$)

2.10 (s, 3H (-CH_3)); 2.48-2.56 (t, 2H(-CH2)); 3.00 (bs, 2H (-SCH_2)); 3.77 (bs, 4H(-C=CH_2, -CH-CH-)) 4.47 (bs, 2H (-NH_2)); 4.77 (bs , 2H (-CH-,-NH-) 6.67 (s, 1H (aromatic-CH-)).

CEF-3 ^{1}H NMR (DMSO)

1.89-1.98 (d, 2H (-SCH_2)); 3.55-3.59 (m, 1H (-C=CH)); 3.62-3.70 (m, 1H (-C=CH)); 3.99-4.04 (m, 2H (-CH-)); 5.06-5.17 (d, 2H (benzyl –CH_2)); 7.09 (s, 1H (-CH=C)); 7.30 (s, 1H (aromatic –CH)); 7.35 (bs, 5H (aromatic –CH)).

Mass spectroscopy

The characteristics mass peak (m/z)of CEF-1, CEF-2 and CEF-3 were obtained at 409.07 [M + 1], 462.70 [M+ K], 486.1 [M+1] as shown in **Figures 7, 8 and 9** respectively.

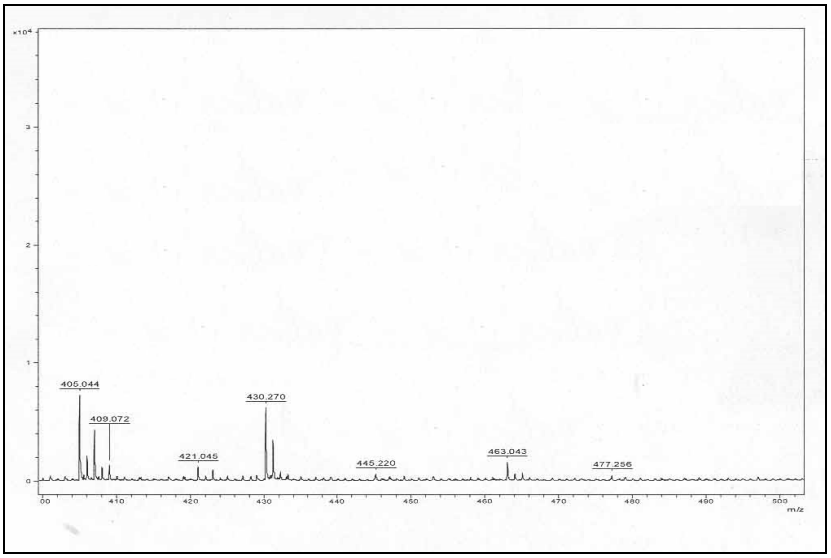

Figure: 7. Mass spectrum of CEF-1

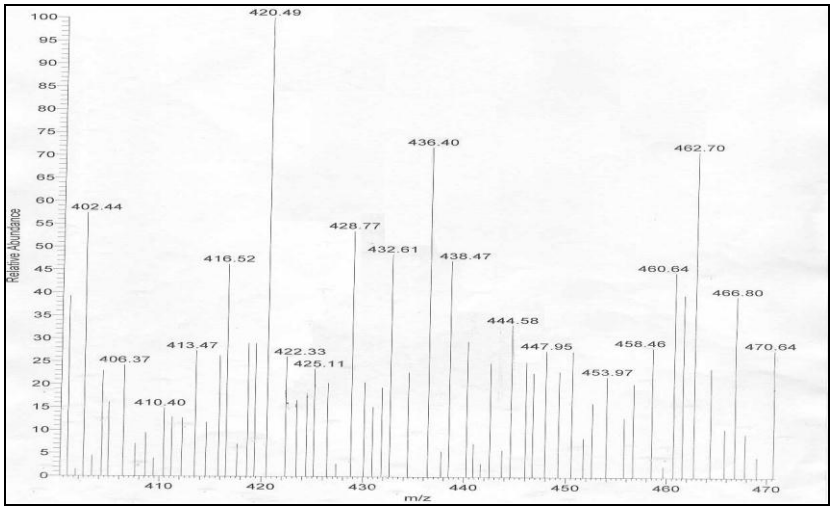

Figure: 8. Mass spectrum of CEF-3

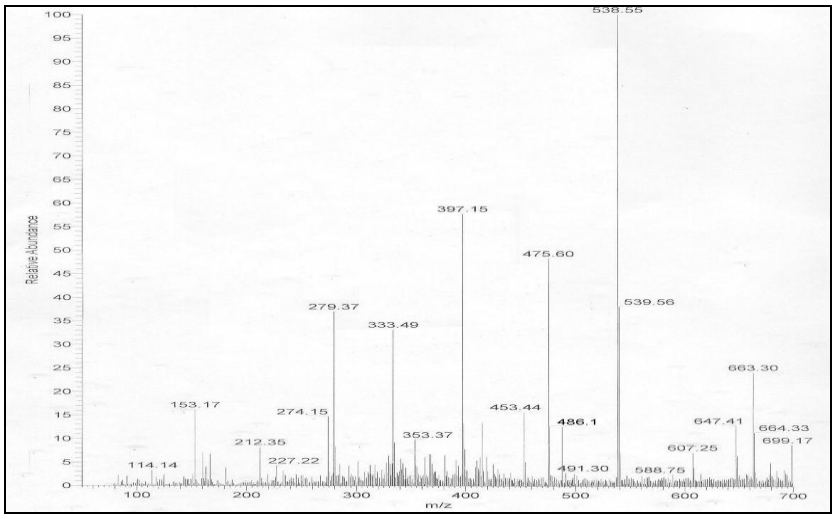

Figure: 9. Mass spectrum of CEF-3

CONCLUSION

Introduction of ester group as a prodrug strategy greatly affects the physicochemical behavior as seen from the data obtained. The solubility of all ester prodrug CEF-1, CEF-2 and CEF-3 is more in acidic media due to only available ionisable amino group. Additionally, all ester prodrugs were found to undergo hydrolytic cleavage under basic condition at an enhanced rate as compared to acidic condition. Additionally the difference between drug and prodrugs were observed in order of lipophilicity. Introduction of lipophilic group i.e. ester into cefdinir enhances the lipophilicity which may warrant the enhancement in permeability across cell membrane. Further *in-vivo* study of the cefdinir and CEF-1, CEF-2, CEF-3 will be carried out to compare the difference in bioavailability of cefdinir and prodrug respectively.

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