3. Materials and Methods

3.1 List of Instruments

- **BRUKER ADVANCE II 400.** Spectrometer was taken in recording of H-N.M. R. of analyzing compound utilizing tetra methyl silane (T.M.S) as I.S or internal standard, Di methyl sulfoxide is solvent (DMSO).

- Mass Spectra were measured in a By Mass Spectroscopy: Agilent Triple Quad System L.C-M.S. – M.S. System 6410) electronic impact technique was used with Electronic ionization =70 e.V & D.M.K. 400 V. pH reading had been taken in solution of H₂O on ten percentage weight by volume at twenty five degree Centigrade in a Crison micro pH 2001.

- The IR spectra had been recorded by using PERKIN ELMER-FTIR-SPECTRUM 100 by using KBr pellets.

- The reaction monitoring and purity was done by HPLC by using LC-2010CHT SHIMADZU by using BDS-hypersil (250×4.6mm×5µ).

- The reaction monitored was also done by GCHS PERKIN ELMER CLARUS 500 ,TURBOMARIN 16 by using G-43 column (30×0.53mm×3µ).

5.2 List of Chemicals used & their manufacturer:

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Grade</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACETONE</td>
<td>B (For Synthesis)</td>
<td>SPECTROCHEM</td>
</tr>
<tr>
<td>ISOPROPYL ALCOHOL</td>
<td></td>
<td>Central Drug House (P) Ltd.</td>
</tr>
<tr>
<td>TPP</td>
<td>A(For Synthesis)</td>
<td>MERCK</td>
</tr>
</tbody>
</table>
SODIUM CHLORIDE A (For Synthesis)  MERCK
METHANOL B (For Synthesis)  SPECTROCHEM
PYRIDINE Alpha Chemika
SULPHURIC ACID B (For Synthesis)  SPECTROCHEM
MDC B (For Synthesis)  SPECTROCHEM
ACETALDEHYDE B (For Synthesis)  Alpha Chemika
SODIUM METABISULPHITE MERCK
CARBON A SPECTROCHEM
SODIUM IODIDE A (For Synthesis)  MERCK
TRIMETHYLCHLOROSILANE A (For Synthesis)  SPECTROCHEM
HEXAETHYL DISILAZINE A (For Synthesis)  SPECTROCHEM
SODIUM BICARBONATE Laboratory Grade SPECTROCHEM
DIMETHYL FORMAMIDE A (For Synthesis)  SPECTROCHEM
IMIDAZOLE A (For Synthesis)  SPECTROCHEM
METHASULFONIC ACID SPECTROCHEM
METHYL MORPHOLINE For synthesis SPECTROCHEM
AMMONIA B (For Synthesis)  SPECTROCHEM
SODIUM HYDROXIDE B (For Synthesis)  Qualigens, Fine Chemicals, Mumbai
TOLUENE A (For Synthesis)  SPECTROCHEM
HYDROCHLORIC ACID Central Drug House(p) Ltd. New Delhi
<p>| | | |</p>
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<th></th>
<th></th>
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<td>SPECTROCHEM</td>
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<tr>
<td>SODIUM CARBONATE</td>
<td>Laboratory Grade</td>
<td>SPECTROCHEM</td>
</tr>
<tr>
<td>SODIUM BICARBONATE</td>
<td>Laboratory Grade</td>
<td>SPECTROCHEM</td>
</tr>
</tbody>
</table>
3.3 METHOD:

Synthesis of cefprozil:

**First Step:** Convert para-methoxy benzyl-seven-phenyl acet amido-three-chloro Me-3-cephem-4-COO⁻ into of para-(CH₃O) benzyl-7-C₆H₅acetamido-3-(Z or E-CH₂=CH-CH₂⁻)-3-cephem-four-COO⁻.
Firstly, check the round bottom flask, it should be clean and dry. Added para-methoxy-C₆H₄-CH₂--7-C₆H₅-lacetamido-3-ClCH₂-3-cefem-4-carboxylate salt (50gm) in to round bottom flask, methylene chloride (300ml) and water (300ml), sodium bromide(40gm), sodium iodide (8gm) and tiphenylphosphine (TPP) (28.5gm) are added at 25°C and contents are stirred for 1 hour 30 minutes at 25°C.after 1 hour stirring send draw the sample from the reaction mass and send the sample to analytical check the GCLE content of HPLC.GCLE content should not more than 1%.If the reaction does not comply continue reaction for further & send sample for HPLC upto completion of reaction. Then the layers are separated, the resulting organic layer is cooled to 0°C and then chilled 1.5% sodium hydroxide solution (300ml) was added. 

The contents are agitated for two hours at 0°C. Draw the sample from the reaction mass & send sample to analytical to check purity of complex for informative only. TPPC content not more than 1% , separated the layers and washed resulting organic layer with saturated sodium chloride solution(12ml).Then the organic layer is cooled to -10°C, isopropyl alcohol (IPA) (350ml) water(150ml) and aqueous acetaldehyde (45.33gm) are added into RBF and stirred for 8 hours. The reaction mass is treated with sodium metabisulfite. 

Stirred for 10 minutes and separated. Distilled off methylene chloride, isopropyl alcohol(200ml) and water (100ml) are added to the reaction mass and agitated for two hours at zero to five degree centigrade .then the separated solid is filtered and dried under vacuum to give p-methoxybenzyl-7-phenylacetamido-3-(Z/E-propenyl)-3-cephem-4-carboxylate. The completion of reaction was monitored by HPLC. The product was filtered& dried and used in next step.
**Second step:** interchange of p-methoxy benzyl-7-phenyl -CH$_2$CONH$_2$-3-(cis/trans-propenyl)-3-cephem-4-carboxylate to 7-NH$_2$-3-(cis/trans-propenyl)-3-cefem-4-carboxylic acid.

Check the round bottom flask it should be dry and clean. Phosphorous pentachloride (30gm) is added to methylene chloride (71.69ml) at 25°C to 30°C, cooled to 15°C under nitrogen pressure and then pyridine (9.92gm) is added for thirty minute at 15-20 degree centigrade. Contents are agitated to twenty minutes at -12°C. Then para – methoxy phenylmethyl-7-phenylmethylcarboxamido-3-(Z/E-propenyl)-3-cefem-4-COO$^-$ (32.64gm) is added to the reaction mass at -12°C, agitated & mixed to one hour thirty minutes at -twelve degree centigrade, & then cooled to -25°C. 1,3-propanediol (27.57ml) is added to the reaction mass for 30 minutes at -twenty five degree centigrade, mixed with agitation thirty minutes at this temperature and phenol (110.29 ml) in methylene chloride (27.75ml) was gradually mixed at -25 degree centigrade. The rxn mass was vibrated to two hours at -25 deg. centigrade to -15°C. Water (ml) is added at -15 to degree centigrade and centrifuged for 15 minute. Then separated the layer and organic layer is extracted with 2N HCl (450ml). The total aqueous layer is washed with methylene chloride (110 ml), pH is adjusted to 2.0 with 25% NaOH at 0-5°C and stirred for 1 hour at the same temperature. Then the separated solid is filtered and washed with acetone (ml) to give to 7-NH$_2$-3-(cis/trans-propenyl)-3-cefem-4-COOH and use this product in next step.

The completion of reaction was monitored by HPLC.
STAGE: 02

GPRIE + MDC, PCl5, PYRIDINE + 1,2-PROPANEDIOL → 7-APRIE + PHENYL ACETIC ACID

PHENOL + MDC + DM WATER + DIL HCl + NaOH → PARA HYDROXY BENZYL ALCOHOL + 7-APRA
**Third Step:** Conver of 7-NH₂ -3- (cis/trans-CH₂-CH=CH-)-3-cefem-4-carboxylic acid into cefprozil D.M.F. solvate.

**Stage 01:**
Take a clean and dry round bottom flask and to slurry of para-methoxy-benzyl-7-phenylmethyl carboxamide-3-(Zuasomman/Entegegan-propenyl)-3-cephem-4-carboxylic acid (I) (20gm) in methylene chloride (80ml) is added hexaethyldisilazane (13.2ml), timethyl chlorosilane(8.4ml)and imidazole (200gm) and the contents are heated to reflux to four hrs. Then resulting solutn is cooled to -15 degree centigrade under nitrogen atmosphere to give [7-trimethylsilylamino-3-(Zuasomman/Entegegane-popen-1-yl)-3-cephem-4 - COOH] timethylsilyl ester.

**Stage 02:**
MDC (120ml) is added to [R-(Z)]-4-hydroxyl-α-[(3-methoxy-1-methyl-3-oxo-1-propenyl)amino] benzene acetic acid (III) monopotassium salt (29gm), cooled to -20°C and dimethylformamide (106.67ml) is added at this temp. The compound is prepared by the reaction of p- hydroxyphenyl glycine with methyl - 3- oxobutanoate in the presence of isopropyl alcohol in basic mediun (KOH).Then methasulfonic acid (0.23ml) and N-methylmorpholine (0.3ml) are added. The contents are cooled to -50 to -60°C,ethyl chloroformate (14ml) is added and stirred for 1 hour 30 min. N,O-bis(trimethylsilyl) acetamide (26ml) is added to the reaction mass and stirred for 30 min.

**Stage03:**
[7-trimethylsilylamino-3-(Z/E-popen-1-yl)-3-cephem-4-carboxylicacid] timethylsilyl ester is added to this solution. The contents are stirred for 3 hours.2N HCL(80ml) is added and layers are separated. Then the mixture of DMF (300ml) & acetone(75ml) is added to the reaction mass ,carbon(2gm) is added and mixed with magnetic stirrer for thirty minutes. Filtered reaction mass and cleaned with washing of dimethyl foramide of hudred milliliter, 7 pH adjusted to 6-6.5 with ammonia solution. Then the precipitated solid is passed through filter paper, cleaned with D. M. F. & acetone. Vacuum pump is used to dry at 40°C to yield cefprozil DMF solvate, The product was used in the next step. The completion of reaction was monitored by HPLC. The reaction completion was determined with prpartive thin layer chromatography and high pressure type liquid chromatography of reverse phase.
The substrate and products and intermediates are spotted to thin layer chromatographic plates and these are also mixed with proper solvents of mobile phase. The mobile phase is organic or aqueous solvent types. The selection and identification of mobile phase is based on the hit and trial of these solvents blank and with the substrate and intermediates and final products. As the starting material is derivative of 7-cephalanoric acid derivative that is converted to final product cefprozil. There are two intermediate in the process so there are total five spots are monitored in the preparative thin layer chromatography and reverse phase liquid chromatography.

The five spots are shown on the preparative plate and identified separately. Their concentration is identified with help of densitometer on the other hand the high pressure liquid chromatography produces a chromatogram of results. There are five peaks in the results. The peaks are identified with retention time and their relative concentrations were found by calculating the area under the curve. The comparative study was done and the results were discussed. Thus both the techniques RP-HPLC & TLC are very useful for reaction monitoring in the synthesis of products.

The high performance liquid chromatography also is very useful in determining the rate of reaction and there intermediate. On the basis of the results we can interpretate that reaction is going on positive direction or negative direction. There is output is reaction or not, the yield of final product is increased by adding catalysts or changing the solvents of the reaction. This also gives information about which types of reaction is taking place nucleophilic aliphatic addition or substitution, electrophilic aromatic substitution or addition and free radical substitution or addition. Some ideas about the mechanism of action of these reactions can be generated on the basis of final product formation and the yield of the final product.
STAGE: 03

STEP: 01
SILLYLATION OF 7-APRA

\[
\text{7-APRA} \to \text{MDC, IMIDAZOLE} \quad \text{HMDS, TMCS} \quad \text{SILYLATED 7-APRA}
\]

STEP: 02
MIXED ANHYDRIDE

\[
\text{AMOXY DANE SALT} \to \text{MDC, DMF, NMM, MSA} \quad \text{ETHYLCHLOROFORMATE} \quad \text{MIXED ANHYDRIDE OF AMOXY DANE SALT}
\]

STEP: 03
SOLVATED CEFPROZIL

\[
\text{\text{7-APRA} + \text{AMOXY DANE SALT} \to \text{MDC, DMF} \quad \text{DIL HCl} \quad \text{1.5 DMF} \quad \text{SOLVATED CEFPROZIL}}
\]
Step 4: Conversion of cefprozil DMF solvate to cefprozil Monohydrate.

The combined mixing of cefprozil Di Methyl Formamide solvate fifteen g.m and water (thirty milliter) is stirred for 1 hour, filtered and washed with acetone (30ml) & vacuum pump is used to dry the product 40 -50 degree centigrate to give cefprozil monohydrate containing ten percentage E-isomer. The completion of reaction was monitored by High Pressure Liquid Chromatography. The final product is collected.

Melting point determination:
The determination of melting point of synthesized product is done with open capillary method. The product is filled in the capillary that is sealed from one side with help of ignition of capillary one and sealing after melting. The product is filled in the capillary. This capillary is then tied with the thermometer of the apparatus or directly to the thermometer. Then the apparatus is started and the reading is noted then the product starts to melt and second reading after complete melting. The range of melting point is denoted. Other method is that the capillary with thermometer is kept into the theil tube or open apparatus or beaker with liquid paraffin oil. The oil is heated on hot plate or heating mental. When the product is started to melt the temperature is noted and after complete liquefaction second reading is noted and melting range is decided.

3.5 Solubilities Studies:
The solubility profile of compounds are given in Merck Index via using these solvents analyze the solubility of compounds by dissolving 10 mg each of the compound in given solvents.

3.6 Determination of Reaction Monitoring:
Reverse phase High Pressure Liquid Chromatographic analyses of rxn monitoring were carried out on SHIMADZU LC2010CHT by using following condition in various step of reaction all these condition are as USP : the following steps were carried out.

**Stage 1**: The scheme a condition used for reaction monitoring from GCLE to GPRE:
Stage 1: Column inertsil C-18 ODS(250×4.6mm×5µ),oven temp-30°C, run time 40min.i nj. volume-10µL, λmax- 220 nm,Buffer Preprn: 2.8012g (Na₂HPO₄) dissolved in one liter of H₂O pH=4.5 was obtained with O-phosforic acid (H₃PO₄),Organic modfer: acetonitrile, Mobile phase-buffer, marker solution was prepared by dissolving 2.5 gm of GCLE and 12.5 gm of GPRE & 2.5 gm of TPP into 50 ml of diluent(ACN50). The retention time of following salts are as follows, which are expected entity of the reaction of GCLE and GPRE.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCLE</td>
<td>41.87 min.</td>
</tr>
<tr>
<td>TPPC</td>
<td>4.56 min</td>
</tr>
<tr>
<td>GPRE</td>
<td>14.42 min</td>
</tr>
</tbody>
</table>

**Stage 2** condition used for reaction monitoring from GPRE to 7-APRA:
Stage-2 Column: inertsil ODS C-18(250×4.6mm×5µ),oven temp. 30°C,λ max – 220nm,rate of mobile phase –one mili liter per minutes, injection volm –ten ml.,run time 40 min, buffer prep’n - 0.2 M of (Na₂HPO₄) dissolved in 1000 ml, p H is done 7 by using orthophosphoric acid.Mobile Phase A (Buffer: ACN 45:55) diluent (Buffer: Mobile Phase A 90:10).sst solution was prepared by dissolving 12.5 gm 7-APRA,5.0 gm
GPRE, 5.0 gm APRE and 5.0 gm of Phenol in 50 ml Of diluent. The retention time of following Components are as follows, which are expected entity of the reaction of GPRE to 7-APRA:

**TABLE 4:** Rf value of 7-ADCA, Phenol, NaI, 7-APRA

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rf Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-ADCA</td>
<td>5.08 min.</td>
</tr>
<tr>
<td>PHENOL</td>
<td>24.61 min.</td>
</tr>
<tr>
<td>NaI</td>
<td>3.47 min.</td>
</tr>
<tr>
<td>7-APRA</td>
<td>6.54 min.</td>
</tr>
</tbody>
</table>

**Stage 3:** Condition used for reaction monitoring from 7-APRA to cefprozil.

Stage 3 column -ODS- hypersil (250×4.6mm×5µ), oven temp. – 30°C, λ max – 220nm, flow rate – 10 ml/min, inj. volume –10 ml, run time – 40 min, Buffer prep’n (mobile phase A) – Ammonium dihydrogen phophate 11.5 gm in 1000ml of water. Mobile Phase, Diluent = buffer, mobile phase B (Buffer : ACN 50:50), sst was prepared by 3.0gm of cefprozil & 6.0 gm of 7-APRA.

The retention time of following Components are as follows, which are expected entity of the reaction of 7-APRA TO CEFPROZIL:

**TABLE 5:** Rf value of 7-APRA, CZLN-Z, CZLN E.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-APRA</td>
<td>6.54 min.</td>
</tr>
<tr>
<td>CZLN-Z</td>
<td>23.88 min</td>
</tr>
<tr>
<td>CZLN-E</td>
<td>26.49 min</td>
</tr>
</tbody>
</table>

**INTERPRETAION OF SPECTRA OF NUCLEAR MAGNETIC RESONANCE**

**NUCLEAR MAGNETIC RESONANCE KEY POINT**

Fundamental:
In the magnetic resonance the sample is being contacted with high mega herz radiofrequencies. After that a manget is swept near the sample or vice versa. The sample protons or C-13 carbons aligned with or opposed the magnetic field. If these protons aligned with they absorb the radiaofrequency energy and setup resonance that is known as nuclear magnetic resonance. The absorbed energy is measure in the form of chemical shift in parts per million. This the basic fundamental principle of nuclear magnetic resonance.

**Application in Pharmaceutical Analysis:**

- We can determine the exact protons and carbons with the help of H-NMR & C-13 NMR in starting material and final product.
- Nuclear magnetic technique is also very useful to estimate impurities in the products and enantiomer forms of the synthesized products.
- Finger printing region is easily identifidied with the help of NMR
- Analysis of sample quantitatively done with the help of proton nuclear magentic resonance and carbon thirteen nuclear magnetic resonance.

**Strength**

- This is very important tool providing information about the structure of the atom without any difficulty. The molecule can be easily interpreted for their types of hydrogen and carbon atoms like primary, secondary, tertiary or may be quaternary.

**MASS Spectroscopy Key Points**

**Principle**

Mass spectroscopy is based on the identification of mass charge ratio of the generated ions. The sample is inserted into ionization chamber by the probe it may be gas, liquid or solid. The sample is bombarded by the heavy energy electrons. These electrons break down the product into fragments of parent and daughter ions that are movable to accelareted cathods through the slit after passing through electrical field these are entered into the magnetic field where the centripital force is equal to centrifigal force. The ions are moved to magnetic field and m/e ratio is founded and interpreted.
Applications

- This spectroscopic technique is very useful for the structure elucidation of the known synthesized compounds by determining its molecular weight from its parent ions and molecular formula with the help of isotope study.
- Gas chromatography and Mass spectroscopy is combinedly known as hyphenated GC-MS technique for determining impurities of the synthesized products.
- Reverse phase high pressure liquid chromatography is combined with Mass spectroscopy is known as LC-MS similar to GC-MS both techniques are very helpful to determine the metabolites in the biological fluids.
- Reproducibility of result is major advantage of reverse phase high performance chromatography.
- Reverse phase liquid chromatography is most versatile and intensive technique at present.
- There is great choice of column selection in the reverse phase high pressure liquid chromatography.
- In the gas chromatography sample is heated to rise the chromatograph temperature and sample may be degraded due to heat by in the high performance liquid chromatography there is no need to heat the sample.

Mass Spectrum had been received in a By Mass Spectroscopy Agilent Triple Quad System LC-MS-MS System 6410) the technique is base on impact of electron with ionization potential of seventy electron volt (eV) D.M.K. 400 Voltage pH readings had been performed in water soln with 10 percent wt by vol at 25 degree centigrade in a Crison micro pH 2001.

Furier Transfer Infra Red Spectroscopy Key Points

Principle
Electromagnetic radiation ranging between 500 per cm and 4000 per centimeter (2500 & 20000nm) is goes through a sample this energy is absorbed by different bond of pi and sigma. This energy make the change of bond between two atoms that is calculated on the basis of Hook’s rule. The frequency of energy absorbed by this bond is measured in the form of wave number per centimeter and being shown by troght in the
infra red spectra. The spectra is recorded between transmittance and wave number. This spectra gives information about different functional groups and finger print region.

**Applications**

- The identification of raw material is done on the basis of fingerprint region of fourier transform infra red spectroscopy.
- FT-IR is very useful in the structure elucidation of unkwon synthesized compounds and natural products. This types of spectra gives infromation of different functional groups in the structure of known products such carbony group, hydroxyl group, phenolic group, halogen groups, alkyl groups, ether groups etc.
- The infra red spectra is also used for the checking purity of the samples.
- Polymorph of the medicaments can be studied with the help of fourier transform infra red spectroscopy.

**Strength**

- The finger print of each compound is unique that may be very useful to authecate the origanility of the compounds and purity of sample.
- Matching of the scanned spectra with orginal in computer software is very useful technique for analysis.
- The phenomenon causes stretching and bending of the bonds in the structre that can cause two types of phenomenon is tretching
  1) symmetric stretching in one way
  2) asymmetric stretching in different directions.
- The carbonyl compounds gives peaks around 1700 to 1800 per centimeter so identification is easy.
- Wave number may be affeted by hydrogen bonding of compound with solvent that weaken the bond and reduce the wave numbers like alcohols and phenols.
- The ketone and aldehydes are identified on the basis of doublet that is found in the aldehyde not in ketones.
- The bending is generally four types two are in plane rocking and scissoring.
- Other types bending is out of plane bending that is wagging and twisting.
- The umbrella type of stretching is very useful to study bending of alkyl groups.
6 REACTION MONITORING CHROMATOGRAMS OF CEFPROZIL

6.1 1st stage GCLE TO GPRE Reaction Monitoring Graph.

Figure 3: I.R of GPRE
Figure 4: Reaction Monitoring Chromatogram
Figure 5: Reaction Monitoring Chromatogram
Figure 6: Reaction Monitoring Chromatogram
**Figure 7**: Reaction Monitoring Chromatogram

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Name</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Area %</th>
<th>RRT</th>
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<tbody>
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<td>11643</td>
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<td>0.57</td>
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<tr>
<td>4</td>
<td>TPPC</td>
<td>4.60</td>
<td>18071716</td>
<td>97.20</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
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<td>5.03</td>
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<td></td>
<td></td>
<td>18592466</td>
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<td></td>
</tr>
</tbody>
</table>
**Figure 8**: Reaction Monitoring Chromatogram
Figure 9: Reaction Monitoring Chromatogram
Figure 10: Reaction Monitoring Chromatogram
Figure 11: I.R of GPRE
6.2 REACTION MONITORING CHROMATOGRAMS OF CEFPROZIL
STAGE 2: GPRE TO 7-APRA

Figure 12: Reaction Monitoring Chromatogram
**Figure 13:** Reaction Monitoring Chromatogram

<table>
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<tr>
<th>Peak#</th>
<th>Name</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Area %</th>
<th>RRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7-APRA-Z</td>
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<td>9516426</td>
<td>90.79</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>7-APRA-E</td>
<td>8.85</td>
<td>965326</td>
<td>9.21</td>
<td>1.35</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1048252</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>
Figure 14: Reaction Monitoring Chromatogram
**Figure 15**: 7-APRA Purity

**Chromatogram**

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Name</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Area %</th>
<th>RRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 ADCA</td>
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<td>128351</td>
<td>0.54</td>
<td>0.45</td>
</tr>
<tr>
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<td>3</td>
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Figure 16: I.R of 7-APRA
Figure 17: Mass Spectra of 7-APRA
6.3 REACTION MONITORING CHROMATOGRAMS OF CEFPROZIL
STAGE 3: 7-APRA TO CEFPROZIL DMF SOLVATE

Figure 18: Reaction Monitoring Chromatogram
**Figure 19: Reaction Monitoring Chromatogram**
Figure 20: Reaction Monitoring Chromatogram
Figure 21: Mass Spectra Of Cefprozil
METHOD DEVELOPMENT

The planned work describe in the following lines:

1. Exhaustive literature survey of cephalosporin antibiotics and its derivatives for various biological activity.
2. Synthesis of cephalosporin antibiotics for process development

3. Determination of Physico-chemical properties of the synthesized compounds by-
   i) Melting point determination
   ii) Solubility profile

4. Recrystallization of synthesized compounds for purification.

5. Structure elucidation of synthesized compounds by –
   1) HPLC – For reaction monitoring
   2) I.R. – For functional group determination.
   3) N.M.R.- For proton determination in synthesized compounds
   4) Mass spectral analysis for molecular weight and molecular formula determination of new compound

**METHODOLOGY**

**Synthesis of cephalosporin antibiotics (for example cefprozil)**

**Step 1:** Conversion of 3-chloromethyl-para-methoxy-C7H7-7-C6H5-acetamido – 3 - cepem-4-carboxyl ester into of 3- chloromethyl-para- C7H7-7-C6H5-acetamido-3- (cis/trans-propenyl)-3-cepem-4-carboxyl ester

**Step two:** Conversion of p-methoxy C7H7-7-C6H5-acetamido-3-(Z/Ente-propenyl)-3- cepem-4-carboxylate to 7-NH2 – 3 - (Z/E- propenyl)-3-cepem-4-COOH.

**Step 3:** Conversion of 7-NH2 - 3 - (cis/anti-propenyl)-3-cepem-4-COOH into cefprozil DMF solvate.

**Step 4:** Conversion of cefprozil DMF solvate to cefprozil Monohydrate.

**Determination of Melting Point Range:**

Prepared products melting point was being estimated by open capillary procedure using melting point apparatus. Products had been placed in one end sealed capillary and placed in the cave made for the capillary. Thermometer was placed in the cave. The temperature at which compounds start melting to the temp at which it completely melts, was recorded as the melting point range.

**Solubilities Studies:**
The solubility profile of compounds are given in Merck Index via using these solvents to analyze the solubility of compounds by dissolving 10 mg each of the compound in given solvents.

**Determination of Reaction Monitoring:**

HPLC analyses of reaction monitoring will be performed on SHIMADZU LC2010CHT by using following condition in various steps of reaction all these conditions are as USP Spectra Analysis

**Key Points of NMR**

Very important spectral method of analysis for the characteristic identification of accurate structure of crude material and finished products.

It may be used to estimate other types of particles (impurities), may be enantomeric impurities, before separation down to be nearly ten percentage.

This technique can be useful to fingerprint mixtures.

The formulation can be quantitatively analyzed before separation with nuclear magnetic technique. The NMR techniques are very useful to study the atom that has magnetic movement or I is not zero. For example $^1$H, $^{13}$C, $^7$N, $^{19}$F etc. atoms have magnetic movement so the nuclear magnetic spectra. The atoms like $^{18}$O, $^{12}$C, etc. have zero magnetic movement so we cannot produce nuclear magnetic spectra of these atoms.

The latest supercoiled magnets or some gases like helium, nitrogen are used to generate high magnet to resolve the compound. The high resolution of magnet like 1.4 T, 2.4 T and 5 T are the powerful magnet to produce highly magnified results.

**Key Points of MS Spectroscopy**

Mass spectroscopy gives a procedure of high specificity for determining or estimating correctness of structure of medicaments and crude material utilized in their synthesis and formulation.

MS in combination with GC & or RP-HPLC gives a procedure for identifying impurity in drug & other additives in the preparations.

GS-Mass Spectroscopy and Liquid Chromatography-Mass Spectroscopy provides highly specific and sensitive procedures to estimate medicaments & its metabolite in serum plasma or other types of fluid of body.
Key Points of I.R
Infra red spectroscopy gives characteristics of functional group identification in the elucidation of structure in unknown compounds. The source of infra red spectroscopy is heated nicrome wire, globar wounded on ceramic support. These are heated to generate infra red radiations. The compounds are taken in to the contact. The sample of drugs for infra red spectroscopy is prepared by the help of transparent potassium bromide, sodium chloride, lithium fluoride in the form of gases, liquid films or solid pellates. These are inserted into the chamber. The iradiations are passed through the samples and goes to the detector to analyze the sample. The detectors are two type electric detector or thermonic detectors and electronic type detectors. The golay type detector, bolometer, thermister, thermocouple detectors are based on thermal and electrical properties of the compounds are measured. The results are produced in the form of furior transform infra red spectra between transmittance and wave number. The compound spectra was interpereted and analyzed to identify different functional groups of compounds. The vibration frequency is measured in the spectra and indicated in wave number per centimeter.

PROCESS DEVELOPMENT TO SYNTHESIZE CEPHALOSPORIN ANTIBIOTICS:
The antibiotics are generally fermented products as observed like pencillins are obtained from penicillinium notatum at the first time then to modify the yield of the antibiotics and commercial production of penicillin a newer species was discovered that is penicillium chrysogenum. The cephalosporins are derived from severage funga cephalosporinum as Brotzu founded in nineteen fourty seven. The newer species was discovered to increase the yield of cephalosporins by discovering new fungal acremonium. So the cephalosphorins are obtained from cephalosporinium acremmonium. To increase the activity and production of these antibiotics some synthetic procedures have been developed from the fundamental molecules such as 6-
amino penicillinic acid for penicillin and 7- amino cephalosporanic acid (7-ACA) for cephalosporins.

Semisynthetic pencillins such as ampicillin, amoxicillin, cloxacillin, dicloxacillin, azlocillin, meslocillin, carbencillin, ticarcillin etc all are synthesized from 6 –amino penicillinic acid. The first generation, second generation, third generation cephalosporins are synthesized from 7-amino cephalosporinic acid. Beta lactam rings are very sensitive to beta lactamase, cephalosporinase due to hydrolysis of beta lactum ring.

Synthesis of chemicals and pharmaceuticals, medicinal agents is branch of pharmaceutical medicinal organic chemistry. These all compound are synthesized by selecting the substrates, after that these substrates are solubilize to optimum solvents where reaction is carried out. The proper equipment such as round bottom flask, two necked flask, three nacked flask, heating mental, thermometer, hot plate, microwave own, sand bath, oil bath, air condenser, reflux condenser are set up according to assembly. The reaction is carried out under optium conditions like use fo catalysts, pH, temperature etc. the mechanism of reaction is studied well and reaction is monitored under preparative thin layer chromatography and reverse phase high pressure liquid chromatography.

Core structure of cephalosporin

![Core structure of cephalosporin](image)

The drug cefprozil, cefpodoxime proxetil, cefetamete and cefotaxime sodium all are synthesized from 7-amino cephalosporanic acid. The cefprozil is second generation
cephalosporin while cefotaxime sodium, cefpodoxime, and cefetamet are the third generation cephalosporins. The penicillins are biosynthesize from three amino acids valine, cysteine and aspartic acid. The compound is derivative from the seventh position of amino acid. In the first reaction the amino group is protected from chloromethyl group.

The group is then reacted with phenyl carboxamide and this group is again reacted with benzyl chloride group. These cephalosporins are susceptible to amidase enzyme. These are sensitive to beta lactamase enzyme. So other protective groups are added. For example cefotriazone is longer acting third generation parenteral cephalosporins that also enters into the cerebrospinal fluid. The tetrazoyl group is inserted to increase the duration of action because this group is lipophilic and drug can be administered two daily and also available in dispersible tablet forms.

These are very emergency life saving antibiotics that are synthesize in this research. The main focus is given on the process development. The process is then validated according to latest regulation and guidelines.

**HISTORY**

Giupse Bortzu discovered the culture of cephalosporium acremonium from the dirty water and the extract was found to inhibit the growth of gram positive and gram negative bacteria. The production of antibiotic is increase by preparing a slant of fungal species with the help of special media that is optimum for growth. The antibiotics were tested for their antimicrobial activeiities.
Sir William Donn school of pathology is situated at Oxford university in London. In nineteen forty eight Sir Abraham and his colleagues prepared a culture of fungus and isolated the antibiotics Cephalosporin P, the structure of this antibiotic is resembling with the fusidic acid that is useful antibacterial agent. Cephalosporin N is another discovered cephalosporin antibiotics that is similar to the antibiotic penicillin N.

The antibiotics that is obtained from the fungus cephalosporin C is active against typhoid fever that is caused by gram negative bacilli salmonella typhi. The antibiotic is not resistance to beta lactamase and degraded easily. The different 7-amino cephalosporinic acid derivatives are prepared that are very potent similar to pencillins that are derived from 6-amino penicillinic acid. The first generation cephalosporin cephalaxin, cefuroxil, second generation like cefclor, cefprozil and third generation cephalosporin like cefotaxime, ceftriazone are synthesized and used.
OBJECTIVE OF INVENTION

A) Accordingly, an object of the present invention is to provide a simple method for preparation of cefpodoxime proxetil, cefetamet, Cefotaxime of high purity.

B) Another object of the present invention is to provide a simple, cost-effective method for prep’n of cefpodoxime proxetil of high purity.

C) Yet another aspect of the present invention is to provide a simple, selective, cost-effective method for preparation of high purity and conforming to pharmacopeial specifications of cefpodoxime proxetil, cefotaxime, cefetamet.

D) Another aspect of the present invention is to provide a process for the preparation of number of cephalosporin antibiotics, in which the by-product are removed and the same can be recycled using simple & industrially viable technique.

E) Another aspect of the present invention is to make a procedure which is less time consuming.

F) Last one aspect of the invention is to make cost effective.

WHOLE FAMILY OF CEPHALOSPORIN DRUGS

The various compounds are synthesized by adding different groups on C-4 & C-7 positions. The C-4 groups are added as carboxylic acid ester derivative and seven position is added as many liphophilic and hydrophilic drugs and have greater potency and duration of action. The third generation cephalosporins are most widely synthetic antibiotics such as ceftazidime, cefpodoxime, cefaperazon, cefotaxime sodium, ceftriazone, cefetamet, cefixime are the most widely drugs. These maximum have antibacterial activity against gram negative bacteria as well as gram positive bacteria. The drugs are also useful in meningitis because the drug should enter into the brain for the activity.

R1&R2 are the different groups which are responsible for creating the different pharmacokinetic & pharmacodynamic parameters of different types of cephalosporin drugs. Both groups are liphophilic and hydrophilic in nature and are added according to requirement of pharmacokinetic and pharmacodynamic activity.
**Cephalosporin Antibiotics:**
The nucleous of cephalosporin antibiotics are changed according to spectrum of activity against different microbes for example first generation cephalosporin antibiotics such as cephalexin, cefuroxil, cephazoline, cephapirin, cefalothine all are active against gram positive bacteria. Second generation antibiotics are extended broad spectrum antibiotics such as cefclor, carbfamef, cefmandole, cefuroxime, cefonicid, cefprozil all are more active against gram negative bacteria as compared to gram positive bacteria. Third generation cephalosporin are specially active against gram negative bacteria such as salmonella typhi, nesseria gonarrea and less effective against gram positive bacteria. These drugs have property of crossing blood brain barrier and useful in brain disorders.
The classification of cephalosporin on the basis of generation is mainly based on the practice not on the principle. For example in Japan there are no fourth generation of drug and cefclor is classified as first generation cephalosporin antibiotics as compared to second generation in united states of America. The system is developed on the basis of discovery and practice. The cephalosporins that are discovered in the starting are known as first generation cephalosporin antibiotics. After sometimes second slot comes in the market with some modification and known as second generation cephalosporin antibiotics. After that third generation antibiotic came to the market and last and latest one is fourth generation.

Cefalosporins are spelled by two different ways like cefa- and cepha- according to their pronunciation. The English speaking countries like United States of America, Canada, Australia etc write cepha- in the spelling of cephalosporin first generation antibiotics in place of cefa- while in other European countries like United Kingdom (Great Britain), France, Germany, Astria, Spain, Holand, Portgal write cefa- in spelling of cefalosporins in place of cepha- in first generation cephalosporins. The nucleus of cephalosporin is composed of beta lactam ring and dihydro thiazine six membered ring. After combing the beta lactam and dihydro thiazine are known as cefem bicyclic ring that contain seven numbering. Numbering system of cephalosporin is two types in one type starts from the nitrogen of beta lactam ring and other type of numbering this starts from sulfur atom of thiazine ring system. 7-substituted amino cefem is known as cephalosporinic acid. The ring structure in penam is composed of two ring beta lactam and thiazolidine ring system and combined form is found in penicillin structures. The beta lactamase or cephalosporinase is the main enzyme that catalyze the beta lactam ring and inactivate after opening of the ring system. The drugs develop resistance due to this enzyme that can be stopped by combining beta lactamase inhibitors.

<table>
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<td>1.</td>
<td>Cefadroxil, cephalaxin, cephapirin, <strong>Gram positive:</strong> the drugs are active</td>
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These drugs are mainly active against penicillin resistance bacteria like staphylococcus aerius, streptococcus pneumonia, bacillus subtilis, streptococcal fragilis etc.

These are mainly useful as beta lactamase sensitive the drug amoxaciilin with clavulanic acid can be replaced with cephalaxin syrup or cephalaxin capsules of sporidac of Ranbaxy. The cephapirin drug is available as parental and oral dosage forms. The drug can be started as injection in hospitalized person and ended with tablet dosage forms.

No activity against methicillin-resistant staphylococci or enterococci.

The drug is active against Klebsilla pneumonia, Escherichia coli and protius mirablis gram negarive bacteria but not active against Peudomonas aeruginosa, bacteroids fragilis. The spectrum is increased as compared to penicillins.

Second generation cephalosporins are less effective against gram positive bacteria as compared to first generation.

These cephalosporins are more effective against gram negative bacteria as compared to first generation cephalosporins such as enterobacter aerogens, nesseria species and Haemophilus influenza species. The spectrum of activity of second generation cephalosporins are

2. Cefprozil, Cefclor, cefonicid, cefuroxime, cefitin

Cefuzonam are effective as anti arearobic antibacterial agent as compared to other agents. The above mention drugs cefprozil is used as parental dosage form while cefclor is used as oral dosage form for the treatment of various disease from different type of bacterias.
Carbapenam, loracarbamap, cefoxitin, cefbuperazone are the other antibiotic considered in second generation cephalosporins. These drugs are most effective against gram negative micro organism of aerobic and anaerobic natures like E. coli, Nesseria meningitis, bacteroid fragilis, pseudomonas aeruginosa increased as compared to first generation of cephalosporins specially for gram negative bacteria. Cefuroxime is effective second generation antibiotics against gram negative bacteria particularly meningities in cerebral disorders. Cefmandole is also alternative for meningities and Eschererichia coli for the treatment.

**CEFTAZIDINE & CEFPERAZONE** are most widely used cephalosporin third generation antibiotics against anti pseudomonal activity.

**3. CEFTAMET, CEFCAPENE, CEFDINIR, CEFDALOXIME, CEFODIZIME, CEFOVECIN, CEFDITOREN, CEFIXIME, CEFDAOLOXIME, CEFTIBUTEN, CEFTIOLENE, CEFTIOFUR**

Ceftizoxime (Cefizox), Ceftriaxone (Rocephin). Third-generation cephalosporins with antipseudomonal activity: Cefoperazone (Cefobid), Ceftazidime (Fortum, Fortaz).

The latamoxilab and oxipenam are also considered in third generation cephalosporin antibiotics. In this particularly clavulanic acid is most widely used as beta lactamase inhibitor againt beta lactamase The drugs those comes under third generation cephalosporin antibiotics have specially antipseudomonal activity. These third generation cephalosporin antibiotics are less effective against gram positive bacteria as compared with first and second generation cephalosporin antibiotics like cefotaxime, cefperazone, ceftriazone etc.

These third generation cephalosporin antibiotics are specially active against gram negative bacteria as compared to other first and second generation cephalosporin antibiotics.

The third generation oral and parental cephalosporins are effective against hospital acquired disease (Nosocomial) so these are useful prophylaxis for surgical operations.
resistance bacteria.

The drug ceftriazone can cross the blood-brain barrier and is most widely used in the emergency for example, complicated meningitis and typhoid fever.

The cefotaxime can also enter the CNS and is also useful in different types of disorders. Other drugs are cefperazone, cefixime, etc.

4. Ceftobiprole and Ceftaroline are included in the fourth generation cephalosporin antibiotics. The other fourth generation cephalosporin is cefepime that is most widely used beta lactamase inhibitor in emergencies.

These antibiotics have activity against pseudomonas aeruginosa and have anti-pseudomonal activity. These antibiotics have zwitterion in the structure so these can easily penetrate the cell wall of gram-negative bacteria as compared to other first, second, and third-generation cephalosporin antibiotics.

5. Ceftobiprole, Ceftaroline these are very costly beta lactam antibiotics in the market. These are very effective in...
β-lactamase antibiotics (Mechanism of action)

The beta lactam antibiotics are effective against cell wall of the bacteria mainly gram positive. The gram positive bacteria have a thick cell wall that is essential for bacterial life. The gram positive bacteria cell wall is fifty to sixty molecule thick as compared to gram negative bacteria that are one to two molecule thick. It means the pencillins and cephalosporin antibiotics are specially effective against the bacteria or microbes that contain cell wall.

The function of cell wall in bacteria
1) the semipermeable membrane of the cell wall provide protection against other types of ingredients or harmful substances.
2) These cell wall membrane provide osmotic pressure.
3) The digestion from the host enzyme is avoided by cell wall
4) The cell wall is supportive for penetration of essential component of the bacteria.

The wall of the gram positive bacteria is composed of peptidoglycan layer that contain two parellal layer of glycan and one perpendicular layer of peptide that is cross linked tightly with glycan layer. The glycan layer is composed of N-acetyl muramic acid (NAMA) and N-acetyl glucosamine. There is composition of techoic acid in the cell wall of bacteria. The enzyme trans peptidase and cell wall transaminase (CWT) have important roll in the biosynthesis of cell wall. The peptode is composed of long chain of amino acids that include glycine, alanine, valine and glutamine. The L-alanine is first converted to D-alanine in the thread then it forms D-alalyl D-alanine cell wall threat at one end of the cell wall that is cross linked with N-acetyl muramic acid and N-acetyl glucosamine by the enzyme cell wall tramsminase in the dipeptidoglycan. There are forty to fifty enzymes are involved in the biosynthesis of gram positive bacteria cell wall. The cell wall is very rigid and complex as compared to gram negative bacteria that is thin and complex.

The cell wall is composed of peptide chain of five amino acid (L-alanine-D-glutamine-L-Lysine-D-alanine-D-alanine) these are interlinked of Uridine di phosphate UDP-N-acetyl glucosamine (NAG) peptide and other is Uridine di phosphate N-acetyl Muramic acid (NAM) peptide. This forms the highly cross link dipeptidoglycan. The different
penicillines and cephalosporins are binded to PBP of the cell wall of bacteria. The bacterial cell wall contains penicillin binding proteins in the structure that vary in each bacteria for example Escherechcia coli have seven penicillin binding proteins other bacteria. Various affinities of penicillin binding proteins like PBP 1a & 1b, 2, 3, 4, and 5. The different antibiotics have different binding sites in the structure for example amoxicillin, ampicillin binds to PBP1. Staphylococcus has four pencillin binding proteins in its cell wall. The bacteria E.coli have rodlike structure which can destroy the PBP 2 of beta lactam antibiotics.

The cell of gram positive bacteria is composed of polysaccharide like acetyl muramic acid, and acetyl glucosamine as compared to gram negative bacteria and other constituent is protein or polypeptide. The sugar and muramic acid are found as alternate layer compared to gram negative bacteria then this layer of glucosamine is interlinked or cross linked to amino acid peptide like alanine, lysine and glutamine. The lysine part is attached to these peptide linkage and use the enzyme transpeptidase. The main enzyme is cell wall transaminase as a bisynthesizer of the gram positive cell wall in the bacteria.

The cross linking of the glycopeptides with peptides of amino acid is catalysed by the enzyme transpeptidase that is inhibited by penicillins and cephalosporin β-lactam antibiotics. The peptidoglycan is heteropolymer composed of Uridyl pyrophosphate UDP N-acetyl muramyl pentapeptide. This is composed with UDP-acetyl glucosamine to form a long chain of polymer. This is cross threated with amino acid l-alanine-l-glycine-l-glutamic acid-l-lysine-D-alanine with the enzyme transpeptidase.

The antibiotics penicillins and cephalosporins inhibit the enzyme transpeptidase by acetylation of the peptidase bond of amino acid. The penicillin binding protein is also situated here where the drug binds and breakdown the cell wall of bacteria and likage of inside material to outside and lysis of bacteria the death. The beta lactamase, penicillinase or cephalosporinase is enzyme that is responsible for inactivation of beta lactam ring in penicillin, cephalosporin and other beta lactam antibiotics.
The above mentioned structure of penilline also contain some poles or orfices in the structure. The pores can carry different types of nutrients, minerals, vitamins, ions etc. to the bacteria. These pores are hydrophobic to outside and hydrophilic to inside so some water soluble amino acid can enter in to the bacterial cell. These types of pores are known as porins.

The enzyme cell wall transamidase are also known as penicillin binding protein 1 and the beta lactam antibiotics are bonded to that place in the bacteria. There are mainly five penicillin binding protein are found in the cell and responsible for pharmacological action of the drugs.
There are total seven penicillin binding proteins. The penicillins or cephalosporin bind to PBP 1 or CWT causes lysis of the bacterial cell and particularly effect the enzyme transpeptidase and transamidase. The cephalosporins or penicillins or other β lactam antibiotics binds to PBP 2 (Penicillin binding protein) or transpeptidase are some less effective as compared to PBP 1. Many drugs bind to the PBP 4 to 6 or enzyme carboxypeptidase are poorly effective in their mechanism of action of drugs and these drugs are easily inactivated with the beta lactamase enzyme.

The gram negative bacteria contain more complex outer membrane and periplasm that is not found in gram positive bacteria. The polysaccharide contain a very complex system in gram negative bacteria that can induce septic shock the gram negative bacteria contain peptidoglycan layer that is not so strong as in the gram positive bacteria.
As directed above the figure the antibiotics binds to Penicillin binding protein 1 strongly after entering in the cell wall of the bacteria. This beta lactam inhibit the enzyme transamidase/transpeptidase that causes acylation of the peptide of of D-alanine amino acid and promote leakage of the drug.

The drug causes leakage in the cell wall due to inhibition of the enzyme that cause cross linking and the net result is removal of all fluid and genetic material of cell of bacteria that causes lysis of the cell and rupture the all structure of bacteria and finally death.

**STUDY OF PHARMACOKINETICS**

**Administration**

Maximum beta lactam cephalosprins of first, second, third, fourth and fifth generation are administered by intramuscular and intravenous routes of administration. Cephalexine, cefaclor, cefuroxime axetil, cefixime, cefdinir, cefadroxil, cephradine, ceftibuten are administered orally.

**Distribution:**
Cefalosporine antibiotics are distributed in all body fluid easily. The maximum first, second generation are not able to cross blood brain barrier for treatment of CNS (Central Nervous System) disorders. Cefuroxime, cefotaxime, cefatriazone, ceftizoxime cross the blood brain barrier and are used for the meningitis. The cerebrospinal fluid has high concentration of cephalosporin antibiotics to treat the disease.

**Fate:**
All of the maximum cefalosporins are secreted through glomerular filtration or tubular active secretion. The some drugs like cefaperazone and ceftriazone are excreted in bile then in fecal matter. Other remaining drugs are excreted through the kidney.

**Theurapeutic Uses:**

**1st generation**
First generation cephalosporins are active against gram positive streptococci, staphylococci, gonococci, micrococci, pneumococci, anaerobic streptococcal pyogenes. These are also used against Methicillin Resistance Staphylococcus aureus (MRSA). The first generation cephalosporin antibiotics are also effective some gram negative species such as Proteus mirblis, Escherichia coli, Kellabesilla pneumonia or we can acronym PEcK.

**2nd Generation**
The 2nd generation cephalosporins are more effective against gram negative bacteria and the spectrum of activity is increased also. These are also effective against Hemophilus influenza, Enterobacter and Nesseria gonorrhoeae so the spectrum of activity can be acronym ad HENPEcK. The drug cefuroxime in this generation is able to penetrate cerebrospinal fluid so the drug is useful in meningitis.

**3rd Generation**
The 3rd generation cephalosporins are specially active against gram negative bacteria infections. Third generation cephalosporins are less effective against Streptococcal pneumonia as compared to ampicillin and this is also Methicillin sensitive staphylococcus aureus (MSSA). The drugs are specially effective in meningitis treatments. Cefaperazone and ceftazidime are specially active against Pseudomonas aeruginosa or have antipseudomonal activity. These drugs are also effective against entrobater Serratia marcesans. These third generation cephalosporin antibiotics are
also useful in the treatment of nosocomial infections (hospital acquired syndrome). The third generation cephalosporins antibiotics can be used as prophylaxis in surgical operations.

The drugs like ceftriazone, cefotaxime are drug of choice for the treatment of infection caused by the bacterial species Enterobacter, Klebsiella, Proteus and Haemophilus. The drugs ceftriazone and cefotaxime is drug of choice of non immunocomperise indivusual and childrens with combination of vancomycin and ampicillins. Ceftriazone is first choice of drug in all types of Gonorrhea caused by Nesseria gonorrhea. Peudomonas meningities is treated with ceftazidime in combination of aminoglycosides antibiotics. The ceftriazone is also very useful in the treatment of community acquired pneumonia from pneumococci or Heamophilus influenza.

**4th Generation**

The 4th generation cephalosporins are very effective in nosocomial infection for treatments. The drug cefepime is orally administered for the treatment of hospitaly acquired infections generally in immune compromised patients. These drugs are zweterionic in nature and easily penetrable to the cell wall of bacteria. So the treatment of infection is very easy. The fouth generation antibiotics are very costly.

**AIM OF PROJECT**

The project relates to an improved and cost effective process for the industrial manufacture of following drugs:

- CEFPODOXIME PROXETIL
- CEFETAMET SODIUM
- CEFOTAXIME SODIUM
- CEFPROZIL PROXETIL

More specifically it relates to preparation of products of good quality with high yield and the by products are removed and the same can be recycled using simple industrial and viable method.

The antibiotic cefprozil is 2nd generation cephalosporin and this is obtained from the 7-amino cephalosporanic acid by side chain process.
Cefotaxime is generally prepared from the heterocyclic ring thiazole and further processing with 7-amino cephalosporinic acid.
Cefetamet sodium is similar drug and can be processed with 7-amino cefalosporanic acid.
Cefpodoxime is derivative of oxime ring in the side chain such as cefuroxime in second generation cephalosporin antibiotics.
Three antibiotics (Cefpodoxime proxetil, Cefetamet sodium and Cefotaxime sodium) are synthesized from MAEM intermediate as shown below in the given scheme.
BASIC REACTION SCHEME OF FOLLOWING DRUGS CEFPODOXIME PROXETIL, CEFETAMET SODIUM & CEFOTAXIME SODIUM BY USING COMMON INTERMEDIATE
Synthesis of Cefotaxime Acid

$\text{7-ACA}$

$\text{+}$

$\text{MAEM}$

$\text{CEFOTAXIME ACID}$
Synthesis of Cefetamet Acid

7-ADCA

+ 

CEFETAMET
Synthesis Of Cefpodoxime Proxetil

7-ACA

7-AMCA

+ MAEM

CEFPODOXIME PROXETIL
The drug cefpodoxime is synthesized and developed in the decades of 1980 to 1990 with high yielding procedures. The drug was taken in third generation cephalosporin and approved by Food and Drug Administration (FDA) in nineteen hundred ninety two. The drug is used mainly for the treatment of pharyngitis, tonsillitis, urinary tract infections and very complicated gonorrheal infections. The drug is synthesized from 7-amino cephalosporanic acid with 3-substituent of methoxy methyl. The four position of cefem ring carboxyl group is substituted with (1-isopropoxycarbonyloxy) ethyl group that is known asproxetil. This is prodrug in proxtel form that is converted to active form after administration and the duration of the drug is increased. The drug is indicated in the treatment of otitis, pharyngitis and amigdelitis. The 7-amino group of cephalosporanic acid is reacted with 1-amino thiazole derivative. This is reacted with oxime and methyl oxime is prepared with reaction of 2-amino thiazole derivatives.

Cemplicef, Vantin and Orelox are three brand name of cephalosporin antibiotic cefpodoxime proxetil for oral administration. These brand may be tablet or capsule as solid dosage form. The cemplicef is brand product of Pfizer now known as Pharmacia and Upjohn. The brand Orelox is lanched by the company Sanofi Aventies. The product cemplicef is available for veterinary use only as oral dosage form. The drug is effective against gram positive and gram negative bacteria with broad spectrum of activity.

Zefira is brand name of cefpodoxime that is specially applicable to children without causing side effects.

Agios Pharmaceutical Limited has prepared the drug zefira for the treatment of children. Vanguard Therapeutics limilted has manufactured Cepotuff in fifty miligram and
hundred milligram and two hundred milligram as dispersible tablet. Dr Reddies manufactured fifty milligram and hundred milligram tablets of cefpodoxime proxetil for the use in different diseases. The brand is known as Pecef. Doxicep is manufactured by Lupin for cefpodoxime proxetil and Gudicef is prepared in Nigeria in the name of cefpodoxime proxetil by other company. The switch is prepared and manufactured by Alkem laboratories. The maximum dosage forms are 200 mg and 400 mg.

Method for preparation of 3-methoxy methyl cefem (the Modified cephalosporanic acid nucleous):

The 1st procedure for getting the above product is carried out by alkaline hydrolysis of 7-amino cephalosporanic acid with sodium hydroxide. The hydrolysed product is reacted to produce acetylated product with phenyl acetyl chloride, phenoxy acetyl chloride and benzoyloxychloride as depending the situations given above. The next step is halogination of intermediate with thionyl chloride or SOCl₂ solution. Pyridine is used solvent in this reaction to get the second. This intermediate is reacted with methanol (MeOH) in the presence of BF₃ or boron trifluoride or calcium carbonate (CaCO₃). The finally prepared product is III.
The next scheme 4 is given below that was obtained from second procedure in this procedure the acetoxy group is directly replaced with nucleophile group. The 7-amino cephalosporinic acid with amino group protection is reacted with salt of alkali metal like NaCl, KCl, KBr, NaBr (sodium chloride, potassium chloride, sodium bromide, potassium bromide) or salt of alkali earth metal such as AlCl3, MgCl2, MgBr2, CaCl2 (calcium chloride, aluminum chloride, magnesium chloride) in CH3OH (methanol) diluted with water (H2O) at a seventy degree centigrade temperature. To obtain the highest yield the metal halide (Calcium chloride) is used with the product obtained in fourth scheme.
Second procedure for synthesis of modified cephalosporanic acid nucleus without protecting amino group is illustrated in the fifth scheme. In this method the intermediate is reacted with MeOH with methane sulphonylic acid to yield the desired product. The reaction takes place between 7-amino cephalosporanic acid and methanol.

Synthesis of cephalosporanic antibiotics of third generation
As above three methods have been discussed in scheme third, fourth and fifth for the preparation of desired producte. Procedure two given in the scheme third is more
advantageous to produce the product nearly sixty five percentage but deprotection of amino group is insereted in the production of 7-ACA at the starting because of amino protection. The scheme 2 and 3 are comparable to each other.

**Method for preparation of 2-(2-amino thiazoyl-4yl)-2-Z-methoxy imino acetic acid side Group:-**

In this procedure the modified cephalosporin nucleus as 7-Amino Cef. Acid treated with 2-(2-amino thiazoyl-4yl)-2-methoxyimino acetic acid to obtain the final product of the scheme with acylation reaction. The ethyl or methyl (Me) acetoacetate (VI) is reacted with NaNO₂ (sod. Nitrite) in acetic acid or sulphuric acid to yield VII product in the first step. In second step The prepared intermediate VII is processed for methylation with dimethyl sulphate [(CH₃)₂SO₄] to obtain the intermediate VIII. The third process is reaction with thionyl chloride or sulphnyl chloride to get the product IX by chlorination reaction. The chloride derivative is generated as intermediate and further reaction is carried out with thiourea in the presence of sod. Acetate as a Base in Hantzsch’s cyclization process. The reaction with water to bring hydrolysis of resulting ester in alkali media further subsequent acidification with HCl or hydrochloric acid produces the product XI. This product XI is the amino group is protected with formylation by reacting with formic acid or HCOOH and finally coupling reaction is carried out to ger the desired product XII and this is purified and melting point is determined and the practical yield is calculated and compared with other synthetic procedures. This method is compared with the procedures mentioned in the old and latest literature. The new improvement in the method are very important for the purpose of commercial production of the desired product for the human serving purposes.
\[
\text{vi} \xrightarrow{\text{NaNO}_2, \text{H}^+} \text{vii} \xrightarrow{\text{K}_2\text{CO}_3, (\text{CH}_3)_2\text{SO}_4} \text{viii} \\
\text{ix} \xrightarrow{\text{Thiourea}} \text{x} \\
\text{x} \xrightarrow{1.\text{NaOH}, 2.\text{HCl}} \text{xi} \\
\text{x} \xrightarrow{\text{HCOOH}, \text{Ac}_2\text{O}} \text{xii}
\]

\text{(2-Formylamino-thiazol-4-yl)-methoxyimino-acetic acid}
Iodo ethyl (Isopropoxycarbony oxy) group prepartion Method:-

In the scheme seven as shown below to obtain the final product XVI there are two procedure may be used. In the first procedure the starting material is taken as ethyl chloroformate (C₂H₅COOCl) and this substrated is reacted with chlorine for halogination and subsiquent reaction with isopropyl alcohol in pyridine as a solvent. The intermediate XV is obtained. To get the final product, this intermediate is reacted with sodium iodide in 18 crown-six-ether that is resulted in final product XVI in the scheme or 1- iodo ethyl isopropoxy carbonyl oxy. In the first step there is other trichloro derivative waste product is produced that decrease the yield of halogenated product chlor ethyl chloroformate so to remove this undwated product a new reaction is carried out between phosgene and acetaldehyde to obtain desired product XIV as mentioned below. But the major disadvantage of this reaction is that the phosgene gas is very harmful and we can not use in large quantities. The final product XVI is washed and crystallize to remove the impurites. The product is filtered after crystallization and confirmed with determing the melting point of the product. This final product is then used for further processing.

![Scheme 7](image)

Preparation method for condensation of 2-(2-aminothiazoyl-4yl)-2-methoxy imino and 1-iodo ethyl (isopropoxy carbonyl oxy group to final reaction with modified Cephalosporanic acid nucleus (3-methoxy methyl cefem):-
The condensation of 1-iodo ethy (isopropoxy carbonyl oxy) group and 2-(2-aminothiazoyl-yl)-2-methoxyimino group to central nucleus cephalosporanic acid is done totally different procedure.

There are two schemes have been given to preparation of cefpodoxime proxetil. In the first method (Scheme 8A) the intermediate III is condensed with ethyl iodide group in the presence of dichloro hexyl amine to result is product XVII. The 7-positioned protected amino group is broken with imino chloride procedure. Further acetylation is carried out with actived product XII to obtain product XVIII. In next step deprotection of amino group and reaction with the group produces the product cefpodoxime proxetil. In the second method (Scheme 8B) the intermediate 5 will produce the better result and yield of the product as compared to product 1. In this method the raw material was used and reaction was carried out without protection of amino group.

The drug cefpodoxime proxetil is product in which side group propoxy carbonyl oxy is added to three position of the cefem ring and the seven position is substituted with 2-amino thiazoyl-4-yl-2-methoxy imino group with the acetylation reactions. These two both steps are very important for the drug. The 7 side chain increases the potency of drug to the enzyme carboxy peptidase and the proxetil group will provide the product esterification that will increase the duration of action as ester. The drug is first hydrolyze to parent drug form and then it will be active for the pharmacological action.

As in the scheme 8 given below all the chemical reactions were carried out and the final product was prepared for the pharmacological effect on different microbial species.
Scheme 8
Second method of preparation (cefpodoxime proxetil):

The second method of preparation of drug cefpodoxime proxetil is given in the scheme 9. The desired product is mainly obtained by combining the three fragments as prepared separately. The main fragment is 3-methoxy methyl cefem to which other two fragments are added by protecting the amino group properly. The group that is attached on the seventh position of the amino group is derivative of 2-amino thiazoyl-4-yl-2-methoxy imino acetic acid derivation the amino group of thiazole heterocyclic ring is protected to formyl group. The other group is prepared as isopropoxy-carbonyl oxy is and added to carboxyl group as ethyl iodide reaction.

In the scheme 9 other second method is discussed for the preparation of cefpodoxime proxetil. In which Intermediate XXII is prepared. The intermediate XXII contains already substituted with 7th position of the modified cephalosporanic acid by the reaction of radical 2-)amino thiozoyl-yl)-2-Z- methoxy imino acetyl with the amino group that is protected and the final compound is given to the name XXII. The intermediate is further reacted with compound intermediate XVI (Isoporoxy carbonyl oxy derivative) in the presence of DCA. The reaction is carried out to obtained the intermediate compound XXI as given below in the scheme. The reversal process is also very useful to synthesize the drug cefpodoxime proxetil. The intermediate product XXI is treated with thiourea (2NHCSNH2) that is resulted in final product XX (cefpodoxime proxetil). The process is here reverse becase XXII is prepared first and then reversed to XX that is final product.

The durg is very useful to treat the diseases that are caused by gram negative bacteria and this prodrug antibiotic is also resistant to the enzyme beta latamase that catalyze the beta lactam ring that is cyclic form of amide. The seventh position derivative gives these properties to the drug. This is third generation cephalosporin parenteral beta lactam antibiotics specially effective against many complicated and uncomplicated disorders.
Scheme 9

Cefpodoxime proxetil
The optimum method for synthesizing the drug cefpodoxime proxetil is reseaching continuously and the best method of synthesis is given in the scheme 10 mentioned below. The scheme gives the first reaction of 3-methoxy methyl derivative XXII is reacted with CATMA (2-(2-chloroacetamido-4-thioylyl-2-methoxyimino) acetic acid. This product is reacted with the XXII product to produce XXIV and this is prepared by the method given in the scheme.

In the next step the chloroformyl group is removed by reacting with thiourea to the product XXIV and further next intermediate is obtained as mentioned in the scheme. The intermediate XXIII is treated with 1-iodo ethyl isopropyl carbonate (XVI) intermediate to obtain the final product XX. This is cefpodoxime proxetil and used as a prodrug.
Cefpodoxime Proxetil

CATMACI-

Scheme-10