Chapter -III

Process for manufacture of Cefprozil monohydrate and synthesis of its impurities

3.1- Introduction

3.2 Literature methods for the preparation of Cefprozil monohydrate

3.3 Objective

3.4 Present work

3.4.1 Selection of method

3.4.2 Preparation of Cefprozil monohydrate

3.4.3 Synthesis of Cefprozil impurities

3.5 Conclusion

3.6 Experimental

3.7 References
3.1. Introduction

Cefprozil monohydrate is a semi-synthetic broad-spectrum Cephalosporin antibiotic consisting of 90:10 Z/E isomeric mixture. Cefprozil is an acid-resistant Cephalosporin due to the para-hydroxyphenyl-glycyl substituent at the 7 position. It acts by binding with target protein on the cell wall of susceptible bacteria, leading to inhibition of cell wall synthesis & the death of the cell. Its in vitro spectrum of activity is similar to those of known cephalosporins Cefaclor & Cefuroxime axetil, and it is additionally active in vitro against penicillin-resistant strains of Streptococcus pneumoniae and certain anaerobes including Clostridium difficile. Cefprozil monohydrate was discovered and developed by Bristol-Myers.

Cefprozil is a commercially valuable and therapeutically useful oral cephalosporin antibiotic effective in controlling diseases caused by a wide variety of Gram positive and Gram negative microorganisms.

Because of its therapeutic usefulness and efficient broad spectrum of activity, there is always a need for an improved synthetic process which would result in a product with high purity and yield, with minimum level of impurities, preferably absent, coupled with ease of operation and, more importantly, with low production cost.
3.2 Literature methods

In literature methods, synthesis of Cefprozil has essentially been carried out by amidification of a 7-amino-3-(1-propen-1-yl)-cephem derivative with α-amino-p-hydroxyphenylacetic acid or its reactive derivative.

Hideaki Hoshi, et al.\(^1\) reported the synthesis of Cefprozil monohydrate. It involves use of 7-Amino cephalosporinic acid as starting material, which is converted to benzhydryl-7-amino-3-halomethyl-3-cephem-4-carboxylate. This intermediate on subsequent condensation with D-2-(t-butoxycarbonylamino)-2-(p-hydroxyphenyl) acetic acid in the presence of N,N'-dicyclohexylcarbodiimide (DCC) as the coupling agent followed by Wittig reaction at 3-position. The final deprotection of the carboxy protecting group resulted in the product Cefprozil. The synthetic scheme is illustrated below (Scheme-3.1).
One of the limitations of the process is that it employs DCC, which is toxic, expensive and requires rigorous anhydrous conditions. Also dicyclohexylurea is formed as a byproduct during the process, removal of which calls for several tedious chromatographic purification and isolation steps to be employed to get the product in pure form. The other limitation is to get almost of Z-isomer in greater than 89% which is active pharmaceutical ingredient.

Hideaki Hoshi, Ichikawa, Jun Okumara et.al\textsuperscript{2} reported an alternative synthesis of the Cefprozil by introducing propenyl group at C3 position of Cephem compound (15) by a Wittig reaction of the triphenylphosphoranyl intermediate derived from a 3-chloromethylcephem compound (5) with acetaldehyde using 10 equivalents of lithium halides such as lithium chloride, lithium bromide or lithium iodide to achieve a Z to E ratio of 9:1. The reaction is carried out in dichloromethane and a co-solvent selected form dimethyl formamide or isopropyl alcohol at a temperature 0 to 25°C; yield 71%; reaction time of 20 to 24 hours. The cephem compound (14) is further deacylated using phosphorous pentachloride in presence of a organic base such as pyridine in dichloromethane followed by alcoholysis using 2 moles of 1,3-butanediol at –20°C; followed by deprotecting the carboxy group using 20 equivalents of TFA in anisole at 0°C to yielded the intermediate 7-Amino-3-(propen-1-yl)-3-cephem-4-carboxylic acid (7-APCA,16) which on condensation with D-p-hydroxyphenylglycyl chloride hydrochloride in presence of organic base affording Cefprozil (11).

This method suffers from a limitation in that it utilizes a large excess of expensive lithium halide such as lithium bromide that is not cost effective for an industrial scale production. The synthetic scheme is illustrated below (Scheme-3.2)
Scheme 3.2

1. $\text{NaOH} \rightarrow \text{PhCHO} \rightarrow \text{NaCO}_3 \cdot \text{H}_2\text{O}$

2. $\text{PPh}_3 \rightarrow \text{CH}_2\text{Cl}_2$

3. $\text{CH}_3\text{CHO}, \text{DMF}$

4. $\text{LiBr}, \text{CH}_2\text{Cl}_2$

5. $\text{Boc} \rightarrow \text{HO}$

6. $\text{TFA} / \text{anisole}$

7. $\text{Cefprozil (11)}$
Vuaille; Andre et. al\textsuperscript{3} describe methods for preparation of Cefprozil, which generally comprise reaction of 4-hydroxyphenylglycine with phosgene, followed by addition of gaseous hydrogen chloride to give 4-hydroxyphenylglycine chloride hydrochloride (18). This is further reacted with a suitable 7-amino-3-propenyl-3-cephem –4-carboxylic acid (16) to give the desired Cefprozil monohydrate. The synthetic scheme is illustrated below (Scheme-3.3).

**Scheme - 3.3**

\[
\begin{align*}
\text{HO-} & \text{CH-COOH} \\
\text{NH}_2 & \\
\text{COCl}_2 & \text{HCl (Gas)} \\
\text{H}_2\text{N} & \text{COOH} \\
\text{17} & \\
\text{+ HO-} & \text{CH-COCl} \\
\text{NH}_2\text{.HCl} & \\
\text{18} & \\
\downarrow & \\
\text{HO-} & \text{NH}_{2} \text{.NH}_{2} \text{.AM} \\
\text{COO-} & \text{NH} \\
\text{S} & \text{N} \\
\text{C} & \text{C} \\
\text{H}_2\text{N} & \text{COO-} \\
\text{11} & \text{H}_2\text{O} \\
\end{align*}
\]

**Cefprozil monohydrate (11)**
However, these methods employ toxic and hazardous phosgene and gaseous Hydrochloride, which are difficult to handle on an industrial scale and cause environmental problem.

Usher John. et al. reported the method for preparation of Cefprozil comprising reaction of 4-hydroxyphenylglycine (17) with ethylene glycol to give an ester which is reacted with 7-Amino-3-(propen-1-yl)-3-cephem-4-carboxylic acid (7-APCA, 16), in presence of enzyme, acylase. However, this method utilizes excess amount of the expensive enzyme rendering the method uneconomical. The synthetic scheme is illustrated below (Scheme-3.4)

Scheme - 3.4

\[
\begin{align*}
&\text{HO-} \quad \text{NH}_2 \\
&\text{17} \quad \text{Ethylene glycol} \\
&\text{H}_2\text{SO}_4 \\
&\quad + \quad \text{HO-} \quad \text{NH}_2 \\
&\quad \text{CH} \quad \text{COOCH}_2\text{CH}_2\text{OH} \\
&\quad \text{16} \quad \text{Acylase enzyme} \\
&\quad \text{NH}_2 \\
&\quad \text{H}_2\text{O} \\
&\quad \text{Cefprozil monohydrate} \quad (11)
\end{align*}
\]
Greil, Ludescher J. et al.\textsuperscript{5} given the synthesis of Cefprozil from salt of 7-APCA with amidine and its use in the production The application describes the synthesis of Cefprozil by the reaction of an amidine salt of 7-APCA (20)a mixed carboxylic acid anhydride of a N-substituted-\(\alpha\)-amino-p-hydroxyphenylacetic acid (21). The patent does not comment on the purity or yield of the product. The synthetic scheme is illustrated below (Scheme-3.5).

**Scheme -3.5**

![Scheme -3.5](image-url)
All the above-described methods discussed herein are associated with the formation of varying amounts of impurities, which affect the overall yield and the quality of the product. Also removal of these impurities calls for additional purification and isolation steps, which render the process lengthy and tedious.

Cephalosporin antibiotics carrying the D-α-amino-α-(4-hydroxyphenyl) acetamido addendum at the 7-position such as Cefprozil and Cefadroxil are generally prepared by reacting the respective 7-amino-3-substituted-3-cephem-4-carboxylic acid or its salt/derivative with an activated derivative of 4-hydroxyphenylglycine such as a reactive ester, a reactive amide or a mixed acid anhydride. However, use of reactive amide or esters makes it difficult to obtain the desired product in high purity and yield because of the occurrence of side-reactions as well as racemization.\(^5\)

Hideaki Hoshi, et.al\(^2\) described method of deacylation using phosphorous pentachloride in presence of an organic base such as pyridine in dichloromethane\(^7\) followed by alcoholysis using 2 moles of 1,3-butanediol at –20°C; deprotecting the carboxy group using 20 equivalents of TFA in anisole at 0°C. This method suffers from a limitation in that it utilizes a large excess of expensive trifluoroacetic acid for de-esterification that is not cost effective for an industrial scale production.

Kameyama; et.al.\(^6\) were reported method of deprotecting the amino and carboxy by first treating 1 mole of the compound with 1 to 10 moles of phosphorous pentachloride and 1 to 10 moles of a organic base such as pyridine in a chlorinated
hydrocarbon solvent such as methylene chloride used in an amount 1 to 50 liter per kg of the compound at −30 to 30°C, to produce a imino-(-lactam compound which is converted to compound (16) by treating with a phenol selected from phenol, cresol, chlorophenol, methoxyphenol or naphthol used in an amount of 0.5 to 200 kg per kg of the compound in presence of a lower aliphatic alcohol such as methanol used in an amount of 0.01 to 0.05 kg per kilogram of the phenol used at a temperature between 0 to 50°C.

The method given by Kameyama; Yutaka, Yamada et.al.⁸ suffers in that it uses large excess of phosphorous halide, organic base and solvent leading to a large reaction mass.

Lanz; et.al⁸ have described a method for preparing compound of formula (16) from a carboxy ester of a alkoxy carbonyl or a aryloxycarbonyl protected amino compound of formula (15) in a single step process comprising of treating with a strong acid such as formic acid, trifluoro acetic acid, alkyl or arylsulphonic acid or Lewis acids like Aluminum halides, boron halides, silyl halides in a solvent such as anisole or diethyl ether. The yields of the process vary from 19 to 72%.,

The method uses large excess of trifluoroacetic acid for deprotection of amino and carboxyl groups.

There have been reported many methods for adjusting the Z- to E-isomer ratio in the preparation of 7-amino-3-propenyl-3-cephem-4-carboxylic acid, 7-APCA or
PACA, (16) which is the key intermediate in the synthesis of 3-propenyl Cephalosporin antibiotics such as Cefprozil (11). There have been reported methods to obtain (Z)-enriched Wittig product by using Lithium salts and suitable solvents as mentioned above. Also, there are several reported methods to enrich the (Z)-isomer content of (E) and (Z) mixture of 7-APCA (16) by derivatisation and crystallization, exploiting solubility differences of various salts of the E- and Z-isomers, and chromatographic separations. For example,

Murray A. Kaplan, et.al⁹ described a process for preparing Cefprozil that is substantially free from the corresponding E-isomer. The process involves preparation of the sodium salt of imidazolidinone derivative of a mixture containing Cefprozil and its corresponding E-isomer, and separation of the imidazolidinone derivative isomers based on their differential solubility.

Ludescher, et.al¹⁰ have disclosed a process for preparing (Z)-isomer enriched 7-amino-3-propenyl-3-cephem-4-carboxylic acid (7-APCA, 16) depleting the corresponding (E)-isomer in a mixture of the (Z)- and (E)-isomers of 7-APCA by subjecting a solution of the mixture to adsorption chromatography.

Ludescher, et.al¹¹ provided a process for preparing a (Z)-isomer enriched 7-APCA (16) by reacting a mixture of (Z) and (E) isomers with a lithium, sodium or potassium base, ammonia or amine to form a mixture of the (Z)- and (E)-isomer of the corresponding slats and depleting the (E)-isomer salt from (Z)-isomer salt in a
solvent mixture in which the two isomers have different solubility to recover the enriched (Z)-isomer salt of 7-APCA(16) and converting it to the free acid.

Kumar Yatendra, et.al\textsuperscript{12} have reported a method of preparing (Z)-isomer enriched 7-APCA by reacting the mixture of (Z)- and (E)-isomers of 7-APCA with a ketone in the presence of an inorganic acid such as HCl, HBr, HI, H\textsubscript{2}SO\textsubscript{4} and HClO\textsubscript{4} to form a alkylidene ammonio salt derivative of 7-APCA, obtaining the (Z)-isomer enriched alkylidene ammonio salt derivative of 7-APCA by crystallizing at a temperature between 0 to 30°C. The 7-APCA (16) was regenerated from the alkylideneammonio salt derivative by suspending in water at a pH of 8.0-8.5 to obtain a clear solution and then treating with activated charcoal and acidifying with 6N HCl.

3.3 Objective and strategy
The object of the present work is to synthesize Cefprozil in high purity, substantially free of impurities by a simple and cost-effective industrially feasible method, which comprises preparation of mixed acid anhydride and its condensation with a protected 7-APCA (16).

It is also an object of the present work to provide an improved method of preparation of mixed acid anhydride by selecting the sequence and temperature of addition of the reagents, which will result in minimization of impurities.

The need of simple and cost-effective method for the preparation of Cefprozil in high purity and yield could be met through minimization of the impurities associated
with the reported methods with concurrent improvement in the purity and yield of the product. The steps involved in the reported synthesis of Cefprozil (11) are minimized and the manufacturing cost was reduced by improving yield and reduction of reactant used.

3.4 Present work

3.4.1 Selection of synthetic scheme

During the course of the present work, we have reproduced the process for preparation of Cefprozil as described by Greil, Ludescher J. et al\(^5\). It was found that the preparation of mixed acid anhydride by the method reported by Greil, Ludescher J. et al. and its subsequent reaction with amidine salt of 7-APCA is associated with the formation of impurities in the range of 6-7%.

In the literature methods, Cephalosporin antibiotic such as Cefprozil have been prepared by reacting the mixed acid anhydride with respective 7-amino-3-substituted-3-cephem-4-carboxylic acid or its salt/derivative such as an amidine salt of 7-APCA as reported by Greil, Ludescher J. et al\(^5\). However, use of 7-amino-3-substituted-3-cephem-4-carboxylic acid, its acid salt or an amidine salt reported in these above described methods is found to give the product in low yield due to side reactions of the unprotected 4-carboxylic acid group and 7-amino group.

Hence there is a need to for a protected form of 7-APCA, which will activate the amino group in the 7-position, efficiently protect the carboxylic acid group, which will
not require additional deprotection steps and can be deprotected, in-situ during reaction work-up.

Based on drawbacks of these reported methods for the synthesis of the Cefprozil we proposed the efficient and cost effective industrially viable process, which is mentioned in scheme (3.6)
Scheme: 3.6

R = Diphenyl methyl (GCLH)
R = Paramethoxy benzyl (GCLE)

22

Triphenyl phoshine
Sodium bromide
Sodium carbonate

24

YLIDE

Acetaldehyde
Dimethyl acetylene

3-PROPENYL DERIVATIVE

25

DMA/PC5
MeOH

7-APCA Ester (15)

Anisole/Trifluoroacetic acid

7-APCA (16)

Dane salt
Ethyl chloroformate
N-methyl morpholine
Dimethyl formamide

Cefprozil (11)
3.4.2 Preparation of 4-methoxybenzyl 7-phenylacetamido-3-[(Z/E)-propenyl]-3-cephem-4-carboxylate.

The preparation of 3-propenyl Cephalosporin derivatives can be proceeds through a reaction sequence involving the intermediacy of phosphonium salt and phosphoranylidene intermediates that are fast degrading. By-products are often formed due to decomposition of the intermediates during isolation. Thus, it is difficult to obtain a Wittig product of high purity in good yield. Further, the Wittig reaction generally yield mixtures containing both the (Z)- and (E)-isomers. The Z-configuration of the 3-propenyl groups is related to the activity of 3-propenyl Cephalosporin antibiotics against gram-negative bacteria, hence, there is a need to minimize the undesired (E)-isomer in these antibiotics. For example, Cefprozil has a limit of Z-isomer content in the range of 89 to 94%.

In our objective, we found that, Wittig reaction to introduce the alkenyl group at C3 position can be effectively carried out using less expensive sodium bromide in presence or absence of a catalytic amount of a hydrohalic acid. Carrying out the Wittig reaction in one-pot synthesis improved the yield and purity of the Wittig product.

Reaction of GCLE (22), in presence of sodium halide and triphenyl phosphine in the mixture of Dimethyl formamide and methylene chloride yielded the corresponding phosphonium salts which on reaction with acetaldehyde in presence of sodium hydroxide furnished 4-p-methoxybenzyl-3-propenyl-7-phenyl-acetamidocephem carboxylate (3-PGCLE, 25).
For preparing the phosphonium salt (23), different metal halides such as sodium iodide, sodium bromide, and Lithium bromide were experimented in various solvents such as acetone/isopropyl alcohol/and mixture of DMF and dichloromethane. Based on the cost consideration and quantity required for the reaction, sodium bromide was the metal halide of choice for preparing the phosphonium salts.

Phosphonium salt (23), formation was found to be facile in polar solvent, particularly in DMF with sodium bromide. Other polar solvents such as isopropyl alcohol did not result in the completion of reaction.

With the view to combine, phosphonium salt (23), formation step & the subsequent step (Wittig reaction), the phosphonium salt formation in the mixture of dichloromethane & DMF was selected. Considering the rate and ease of reaction 2:1 ratio of DMF / DCM was fixed for carrying out the reaction.

For converting the phosphonium salt to ylide (24), number of bases such as sodium carbonate sodium hydroxide & sodium bicarbonate have been tried. Since sodium hydroxide gave comparatively better results, sodium hydroxide was gives results in Wittig reaction.

Effect of temperature on the Wittig reaction was studied at various the temperatures from 5-30°C. There was no effect on the yield and quality up to 5-10°C whereas higher temperature (25-30°C) led to the poor yield & more impurity formation.
In the conversion of ylide (24) to 4-p-methoxybenzyl-3-propenyl-7-phenylacetamidocephem carboxylate (3-PGCLE, 25), 20-30% aqueous acetaldehyde solution as well as neat acetaldehyde (95-98%) was tried. Reaction conversion was found better by using neat acetaldehyde, which was enhanced the yield and quality of the product.

Sodium chloride solution (20%) was played important in the Wittig reaction which helps to control the impurity formation and to improve the yield and quality of the product.

For isolating the final product in pure form, dichloromethane was removed at atmospheric pressure and isolation of 3-PGCLE (25) was studied using solvents like methanol, ethanol and isopropyl alcohol. Isopropyl alcohol was found to be a solvent of choice, since use of ethanol and methanol gave a poor quality of product.

The process, which emerged after studying all these parameters, is given as follows,

Reacting the compound (22) with triphenyl phosphine in the presence N,N-Dimethylformamide, sodium bromide and optionally in presence of catalytic amount of a hydrobromic acid to get the intermediate triphenyl phosphonium salt (23) and reacting the triphenyl phosphonium salt with a sodium hydroxide as a base to get the intermediate (24),

Reacting the intermediate (24) with an acetaldehyde (~98%), in the presence of catalytic sodium hydroxide as base at a temperature in the range of 5 to 15°C, to produce a compound of formula (25) with required ratio of Z and E isomer...
3.4.3 Preparation of 7-amino-3-[(Z/E)-propenyl]-3-cephem-4-carboxylic acid

p-Methoxybenzyl-7-amino-3-Propenyl cephem-4-carboxylate, namely 7-APCA ester(15), was prepared by the reaction of p-methoxybenzyl-7-phenylacetamido-3-propenyl cephem-4-carboxylate(25) with base and PCl₅ to give iminochloride which on treatment with methanol resulted the formation of p-methoxybenzyl-7-amino-3-propenyl cephem-4-carboxylate (15). This, on in situ treatment with trifluoroacetic acid followed by precipitation with sodium hydroxide in D.M. Water resulted in the formation of 7- Amino 3-propenyl Cephalosporinic acid (16).

As per our previous experience in Cefixime molecule deacylation of p-methoxybenzyl-7-phenyl-acetamido-3-propenyl cephem-4-carboxylate, combination of DMA/PCl₅ gave the best results.

Since the p-methoxybenzyl-7-amino-3-propenylcephem carboxylate was not isolated, it was directly proceeded to deprotection by in-situ way. Deprotection of 7-APCA ester (15) was tried with different reagents such as TFA / Anisole¹⁴, TFA / DCM¹³, Aluminum chloride anisole¹⁵, phenol¹³ among these all Trifluoroacetic acid / Dichloromethane combination was found to be suitable for the deprotection in terms of low cost of the process.

Deprotection of 7-APCA ester (15) was attempted at different temperature from 5 to 30°C. The rate of reaction is slow at lower temperature [(5-10°C) reaction require ~20 hrs for completion whereas at higher temperature (25-30°C) impurity formation
was more. Better results were obtained at 18-20°C in terms of a yield and quality of the intermediate.

The detailed process that emerged from the optimization of the above parameters is given in experimental section.

The deacylation and de-esterification can be sequentially achieved in one pot by in-situ method. Deacylating the compound of formula (25) using a phosphorous pentachloride, in presence of a Dimethyl aniline and catalytic amount of chlorotrimethyl silane in the presence of dichloromethane as a solvent, followed by alcoholysis using a methyl alcohol at temperature range of −50 to −20°C to yield a compound of formula (15), which on de-esterified using TFA at 20±2°C to get a compound of formula (16).

All these improvements in conjunction have the advantage in providing compound of formula (11) of high quality and in high yields, which moreover is convenient and cost effective.

3.4.4 Preparation of 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-çephem-4-carboxylic acid (Cefprozil monohydrate,11)

In the literature reported methods, wherein the sequence of addition is such that the Dane salt and the acylating agent are added first, the free acylating agent tends to react with the hydroxy group and leads to the formation of impurity (26) in higher quantities.
The method for the preparation of mixed anhydride as described by Greil, Ludescher J. et al\textsuperscript{5}. comprises addition of 4-picoline and Dane salt to a mixture of DCM and DMF at ambient temperature and cooling the suspension to $-30^\circ C$ followed by addition of the acylating agent and agitating the suspension at $-25^\circ C$ to $-20^\circ C$ and cooling it to $-50^\circ C$, followed by its reaction with an amidine salt of 7-APCA(20), then 6-7% impurity is observed in the reaction mass as against the 2.26% impurity observed in the method of present invention.

Hence there is a need to have protected form of 7-APCA, which will activate the amino group in the 7-position, efficiently protect the carboxylic acid group, which will not require additional deprotection steps and can be deprotected in-situ during reaction work-up.

As per the process described by Barnish; et. Al\textsuperscript{16} the mixed anhydride is prepared by adding a chloroformate, such as ethylchloroformate, to a solution of N-protected-4-hydroxy phenylglycine dissolved in an inert organic solvent at a temperature of $-5^\circ C$ to $0^\circ C$ in the presence of a base.
Most of the literature described the methods for preparation of the mixed acid anhydride are associated with the formation of varying amounts of different impurities. For example, during the preparation of mixed the 4-hydroxy group of the Dane salt is likely to react with the acylating agent thereby forming an impurity which further reacts with 7-APCA or its salts to form an impurity (26).

Limitation of the reported methods for preparation of Cefprozil:

i) Utilize toxic and expensive chemicals such as phosgene, DCC and HCl;

ii) Utilize expensive enzyme like acylase; and

iii) Are associated with formation of varying amounts of impurities which give the product in low purity and yield, rendering such methods less cost effective.

Therefore, a need exists for a simple and cost-effective method for the preparation of Cefprozil in high purity and yield. Such a need could be met through minimization of the impurities associated with the reported methods with concurrent improvement in the purity and yield of the product.

In the present work we have found that the mixed carboxylic acid anhydride of a N-substituted-α-amino-p-hydroxy phenyl acetic acid or its salt (Dane salt,21 ) can be prepared by a careful selection of a specific sequence and temperature for addition of the reagents so that it will result in minimization of impurities during product formation.
The effect of varying sequence of addition of reagents during mixed anhydride preparation on the amount of total impurities formed along with Cefprozil was established by the following experimental evidence.

Cefprozil monohydrate is prepared by silylating the 7-APCA with the mixture of hexamethyl disilazane and trimethylchlorosilane in presence of N,O-Bis(trimethylsilyl)acetamide to get silylated 7-APCA, which on condensed with mixed anhydride of p-Hydroxy phenyl glycine dane salt (potassium methyl) and ethylchboroformate. The condensed product was hydrolyzed with dilute hydrochloric acid and subsequent treatment in a mixture of Acetone-Dimethyl formamide to obtain Cefprozil solvate, which on desolvation with the mixture of DM Water and ethyl acetate to furnish the Cefprozil monohydrate.

3.4.4.1 Role of silylating reagent

Silylation of 7-APCA was tried by using the mixture of hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) with various molar ratio such as hexamethyl disilazane (0.65-1.0 mole) and trimethyl chlorosilane (0.03-0.82 mole). All combination of reagents almost gave similar results, hexamethyl disilazane (0.80 mole) and trimethyl chlorosilane (0.82 mole) is choice of the combination. Results of the various experiments are listed in Table -3.

3.4.4. 2 Effect of catalyst on Silylation of 7-APCA

Different catalyst were attempted in Silylation of 7-APCA such as p-Toluene sulphonamide, acetamide, imidazole, N,O-Bis(trimethylsilyl)acetamide (BSA). Al
catalyst were gives the similar results. N,O-Bis(trimethylsilyl)acetamide (BSA) is the choice of the catalyst as it is easy to handle at small quantity (Table-4).

Table-3

<table>
<thead>
<tr>
<th>S No</th>
<th>HMDS (moles)</th>
<th>TMCS (moles)</th>
<th>Unreacted % 7-APCA in reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.20</td>
<td>0.03</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>0.95</td>
<td>0.80</td>
<td>3.77</td>
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<tr>
<td>3</td>
<td>0.85</td>
<td>0.80</td>
<td>1.15</td>
</tr>
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<td>4</td>
<td>0.80</td>
<td>0.65</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>0.80</td>
<td>0.82</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>0.70</td>
<td>0.72</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Table-4

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Catalyst</th>
<th>Unreacted % 7-APCA in reaction</th>
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<tbody>
<tr>
<td>1</td>
<td>p-Toluene sulphonamide,</td>
<td>1.15</td>
</tr>
<tr>
<td>2</td>
<td>Acetamide,</td>
<td>2.11</td>
</tr>
<tr>
<td>3</td>
<td>Imidazole</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>N, O-Bis (trimethylsilyl) acetamide</td>
<td>1.0</td>
</tr>
</tbody>
</table>
3.4.4.3 Effect of various reagents on the preparation of mixed anhydride Mixed anhydride of p-Hydroxy phenyl glycine dane salt (potassium, methyl) was prepared by ethylchloroformate in the mixture of dichloromethane-dimethyl formamide and N-Methyl morpholine as a catalyst.

3.4.4.4 Role of Dimethyl formamide

The quantity of dimethyl formamide varied from (2 to 4.5 V/g 7-APCA) in the preparation of mixed anhydride. Dimethyl formamide (3.5 V) was found to be suitable for the reaction (Table-5a).

Mode of addition of dimethyl formamide plays important role in the preparation of mixed anhydride to control the carbonate impurity. Addition of dimethyl formamide before (temp 20-25°C) Dane salt reduces the carbonate impurity where as addition of dimethyl formamide after (−50 to −55°C) Dane salt enhance the carbonate impurity (Table-5b).

Table-5a

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Dimethyl formamide Volume/gm 7-APCA</th>
<th>Unreacted % 7-APCA in reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>1.15</td>
</tr>
<tr>
<td>4</td>
<td>3.50</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>3.80</td>
</tr>
<tr>
<td>6</td>
<td>4.5</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Table-5b

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Mode of DMF addition</th>
<th>% Carbonate impurity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Before Dane salt</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>After Dane salt</td>
<td>0.41</td>
</tr>
</tbody>
</table>

3.4.4.5 Effect of base catalyst

Different base catalyst were tried in the preparation of mixed anhydride like Di-sodium hydrogen ortho phosphate, N, N-Dimethyl amino pyridine, pyridine and N-methyl morpholine. Out of these N-methyl morpholine is a suitable catalyst for the completion of reaction and it is optimized to 1% (Table-6)

Table-6:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Base catalyst in % w.r.t. 7-APCA</th>
<th>Unreacted %7-APCA in reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Di-sodium hydrogen ortho phosphate (20%)</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>N,N-Dimethyl amino pyridine (1.2%)</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>Pyridine (2.0%)</td>
<td>9.88</td>
</tr>
<tr>
<td>4</td>
<td>N- methyl morpholine (0.6%)</td>
<td>3.8</td>
</tr>
<tr>
<td>5</td>
<td>N- methyl morpholine (0.8%)</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>N- methyl morpholine (1.0%)</td>
<td>0.25</td>
</tr>
<tr>
<td>7</td>
<td>N- methyl morpholine (2.1%)</td>
<td>2.2</td>
</tr>
<tr>
<td>8</td>
<td>N- methyl morpholine (2.5%)</td>
<td>1.15</td>
</tr>
<tr>
<td>9</td>
<td>N- methyl morpholine (5.0%)</td>
<td>41.0</td>
</tr>
</tbody>
</table>
3.4.4.6 Effect of ethyl chloroformate

Ethyl chloroformate in different molar ratio were attempted from 1.10 to 1.18 mole, better conversion of the reaction obtained in 1.12 mole. Results are shown as below (Table-7)

Table-7

<table>
<thead>
<tr>
<th>Quantity of ethyl chloroformate in mole w.r.t. 7-APCA</th>
<th>Unreacted % 7-APCA in reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.10</td>
<td>3.91</td>
</tr>
<tr>
<td>1.12</td>
<td>1.7</td>
</tr>
<tr>
<td>1.15</td>
<td>3.77</td>
</tr>
<tr>
<td>1.18</td>
<td>1.15</td>
</tr>
</tbody>
</table>

In the present work, the preferred sequence of addition is such that the acylating agent and base are mixed first so that they form a complex. Dane salt is then added so that the acylating agent-base complex reacts preferentially with the carboxylic acid group and very little amount of the free acylating agent is available for reaction with the hydroxyl group and hence results in reduced quantities of impurity (26) as well as other impurities.

(i) If the sequence of addition of the reagents during mixed anhydride preparation is altered in such a way that the Dane salt was first added to a mixture of dimethyl formamide and dichloromethane at −50, the temperature of the suspension was raised to ambient followed by addition of ethyl
chloroformate and N-methyl morpholine, then the product Cefprozil is found to contain total impurities to the tune of 4.6%.

(ii) If the Dane salt is first added to a mixture of dimethyl formamide and dichloromethane at $-50$ °C, the temperature of the suspension is raised to ambient followed by addition of N-methyl morpholine and then ethyl chloroformate, total impurities observed, after condensation with disilylated 7-APCA, amount to 2.94%.

(iii) If the sequence of addition was such that the ethyl chloroformate and N-methyl morpholine was added to a mixture of dichloromethane and dimethyl formamide at ambient temperature, the suspension was cooled to $-35^\circ$ to $-50^\circ$C followed by addition of Dane salt, and the mixed anhydride thus prepared is reacted with a silylated derivative of 7-APCA the total impurities are reduced to 2.26 %.

(iv) Further, if the ethyl chloroformate and N-methyl morpholine were added to dichloromethane at ambient temperature, the suspension is cooled to $-35^\circ$ to $-50^\circ$C, Dane salt is added to the cooled suspension, followed by addition of a of a dimethyl formamide to the solution, then the total impurities formed along with Cefprozil are reduced to 0.64 %. The qualitative results as monitored after condensation reaction by HPLC are tabulated herein below.

Effect of sequence of addition of reagents in preparation of mixed anhydride on the level of impurities.
## HPLC monitoring method results Table -7

<table>
<thead>
<tr>
<th>Sequence of addition of ethyl chloroformate during mixed anhydride preparation.</th>
<th>Unconverted starting material %</th>
<th>Product %</th>
<th>Carbonate impurity (26) formed during reaction %</th>
<th>Total impurity formed during reaction, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Dichloromethane, dimethyl formamide, Dane salt, ethyl chloroformate and N-methyl morpholine</td>
<td>5.10</td>
<td>90.3</td>
<td>2.31</td>
<td>4.6</td>
</tr>
<tr>
<td>(ii) Dichloromethane, dimethyl formamide, Dane salt, N-methyl morpholine and ethyl chloroformate</td>
<td>2.97</td>
<td>94.09</td>
<td>1.81</td>
<td>2.94</td>
</tr>
<tr>
<td>(iii) Dichloromethane, dimethyl formamide, ethyl chloroformate, N-methyl morpholine and Dane salt</td>
<td>4.99</td>
<td>92.74</td>
<td>0.3</td>
<td>2.26</td>
</tr>
</tbody>
</table>
(iv) Dichloromethane, ethyl chloroformate, N-methyl morpholine, Dane salt and dimethyl formamide

<table>
<thead>
<tr>
<th></th>
<th>0.56</th>
<th>98.78</th>
<th>0.45</th>
<th>0.64</th>
</tr>
</thead>
</table>

3.4.4.7 Effect of p-Hydroxyphenylglycine Dane salt

Preparation of mixed anhydride was tried by using different quantities of Dane salt from 1.05 to 1.22 moles. Better results were obtained by 1.05 moles (Table-9)

Table-9

<table>
<thead>
<tr>
<th>Quantity of Dane salt in mole w.r.t. 7-APCA</th>
<th>Unreacted %7-APCA in reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.05</td>
<td>1.07</td>
</tr>
<tr>
<td>1.07</td>
<td>4.1</td>
</tr>
<tr>
<td>1.10</td>
<td>3.90</td>
</tr>
<tr>
<td>1.12</td>
<td>1.20</td>
</tr>
<tr>
<td>1.16</td>
<td>1.15</td>
</tr>
<tr>
<td>1.20</td>
<td>3.77</td>
</tr>
<tr>
<td>1.22</td>
<td>2.3</td>
</tr>
</tbody>
</table>

3.4.4.8 Effect of temperature

Preparation of mixed anhydride was carried out at different temperature from −20 to −50°C. No adverse effect of temperature on the reaction up to −20°C but for safer side selected the temperature range between −35 to −40°C Table-10
Table-10

<table>
<thead>
<tr>
<th>Temperature of Mixed anhydride Preparation in °C</th>
<th>Unreacted %7-APCA in reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20 to -25</td>
<td>4.17</td>
</tr>
<tr>
<td>-35 to -40</td>
<td>1.7</td>
</tr>
<tr>
<td>-45 to -50</td>
<td>2.3</td>
</tr>
</tbody>
</table>

3.4.4.9 Role of co-solvent in preparation of Cefprozil DMF solvate

Different co-solvents were tried in the preparation of Cefprozil solvate along with N,N-Dimethyl formamide, such as Isopropyl alcohol, ethyl acetate, dichloromethane and acetone. Acetone was found suitable co-solvent for the preparation of Cefprozil solvate in terms of good filtration rate and yield.

Quantity of acetone was explored from 3-25 times w.r.t.7-APCA. Three time acetone with 12 time N,N-Dimethyl formamide gives better results Table-11

Table -11

<table>
<thead>
<tr>
<th>S.No.</th>
<th>DMF Vol / g 7-APCA</th>
<th>Co-solvent In times</th>
<th>w/w Yield of solvate w.r.t. 7-APCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.0</td>
<td>Isopropyl alcohol -10</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>10.0</td>
<td>Isopropyl alcohol -25</td>
<td>1.80</td>
</tr>
<tr>
<td>3</td>
<td>10.0</td>
<td>Acetone -25</td>
<td>1.70</td>
</tr>
<tr>
<td>4</td>
<td>12.0</td>
<td>Acetone -3</td>
<td>1.80</td>
</tr>
<tr>
<td>5</td>
<td>12.0</td>
<td>Dichloromethane -15</td>
<td>1.63</td>
</tr>
<tr>
<td>6</td>
<td>12.0</td>
<td>Ethyl acetate -15</td>
<td>1.72</td>
</tr>
</tbody>
</table>
3.4.4.10 Preparation of Cefprozil monohydrate from Cefprozil DMF Solvate

Cefprozil DMF Solvate which on desolvation in aqueous media presence of ethyl acetate and acetone gives Cefprozil monohydrate. Better results were obtained in the mixture of ethyl acetate and water. Desolvation of the Cefprozil solvate was carried out in different quantity of water from 1-6 times w.r.t. 7-APCA in presence ethyl acetate and acetone. The mixture of water (3.0 t) and ethyl acetate (1.80 t) is found to be suitable for better yield Table-12

Table 12

<table>
<thead>
<tr>
<th>S.No</th>
<th>D.M. Water Volume/gm 7-APCA</th>
<th>Co-solvent in times</th>
<th>w/w Yield of solvate w.r.t. 7-APCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>----</td>
<td>1.36 (low purity)</td>
</tr>
<tr>
<td>2</td>
<td>1.50</td>
<td>----</td>
<td>1.31</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>----</td>
<td>1.29</td>
</tr>
<tr>
<td>4</td>
<td>3.4</td>
<td>------</td>
<td>1.20</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>------</td>
<td>0.90</td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>------</td>
<td>0.50</td>
</tr>
<tr>
<td>7</td>
<td>3.0</td>
<td>Acetone (1.0 T)</td>
<td>1.3</td>
</tr>
<tr>
<td>8</td>
<td>1.5</td>
<td>Acetone (1.8 T)</td>
<td>1.3</td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
<td>(Ethyl acetate 1.8T)</td>
<td>1.3</td>
</tr>
</tbody>
</table>
3.4.5 Synthesis, isolation and characterization of impurities:

Regulatory authorities all over the world are becoming very stringent about the purity of an approved drug. Especially there is growing concern about the nature and level of impurities present in such molecules. US Pharmacopoeia specifies that the purity of Cefprozil\textsuperscript{17} should be between 90 to 105%. However, most of the reported methods are associated with the formation of varying amounts of impurities and hence there is need to identify the cause of formation and characterization of impurities.

Mainly two process impurities were observed in the Cefprozil during its synthesis in the range of 0.2 to 1.5%, the molecular weight of these impurities were found by LC-MS and from the molecular weight the structure of the impurities were illustrated as shown below structure (26) and (27). Efforts were made for the synthesis of these impurities.
Impurity (26) was synthesized from 7-amino-3- [(Z/E)-propenyl]-3-cephem-4-carboxylic acid and N-substituted-α-amino-p-hydroxy phenyl acetic acid or its salt (Dane salt) by using excess quantity of ethylchloroformate and N-methyl morpholine. as process described in example (5).

Similarly the second impurity (27) was synthesized from silyl protected Cefprozil and mixed anhydride of N-substituted-α-amino-p-hydroxy phenyl acetic acid salt (Dane salt). Detailed process was mentioned in experimental section as example (6).

3.5 Conclusion
In summary, the present work provides a highly selective method for preparation of Cefprozil in high yield and high purity, substantially free of impurities, which is simple, convenient and cost-effective and more importantly does not suffer from the limitations associated with the reported methods.
3.6 Experimental:

Preparation of 4-methoxybenzyl 7-phenylacetamido-3-[(Z/E)-propenyl]-3-cephem-4-carboxylate (25.)

To a suspension of 4-methoxybenzyl 7-phenylacetamido-3-chloromethyl-3-cephem-4-carboxylate (100 g, 0.205 mol) in dimethyl formamide (200 ml), NaBr (24 g, 0.233 mol), triphenyl phosphine (58 g, 0.220 mol) and dichloromethane (100 ml) were added and stirred at 25 to 27°C for 2 hrs. The reaction mixture is cooled to 10°C and dichloromethane (1000 ml) was added followed by addition of aqueous solution of sodium hydroxide (9.6 g in 80 ml) in 45 to 60 minutes at 5 to 7°C. After stirring for 2 hrs, water (1000 ml) was added to the reaction mixture and stirred for 15 min at 10 to 12°C. The organic layer was separated and washed with water (1000 ml). The organic layer was added to a flask charged with 20% w/v aqueous NaCl solution (1200 ml) and isopropyl alcohol (200 ml) cooled to 5-10°C. A solution of aq. NaOH (1.37 g in 35 ml) was added in 10-15 min followed by acetaldehyde (140 ml, 3.2 mol) in 45 min at 5-10°C, stirred for 4 hrs at 5 to 10°C and 10% v/w hydrochloric acid (10.4 ml) was added and stirred for 15 min at 10°C. The organic layer was separated, concentrated to almost no solvent and isopropyl alcohol (400 ml) was added, heated to 50 to 52°C with stirring for 30 min, cooled and filtered the solid. The wet solid was washed with chilled isopropyl alcohol (100 ml) and dried under vacuum at 35-40°C till moisture content was not more than 1% (yield 49 g, 50%, Z/E ratio 92/8, by HPLC)
\[ \text{NH} \quad \text{S} \quad \text{O} \quad \text{O} \quad 25 \quad \text{OCH}_3 \]

$^1$H NMR Spectrum (CDCl$_3$, $\delta$ in ppm):

1.51–1.53 d (3H, (Z)-CH$_3$), 1.75 d (3H, (E)-CH$_3$), 3.18-3.79 m (4H, -S-CH$_2$ and PhCH$_2$), 3.79 s (3H, -OCH$_3$), 4.95-5.13 m (3 H, CO$_2$-CH$_2$ and -COCH-CH-), 5.59—5.82 m (1H, -CH=CH(CH$_3$) and -COCH-CH-), 6.03-6.09 d (1H, -CH=CH(CH$_3$)), 6.86 d (2H, benzene-H) 7.25-7.36 m (7H, benzene-H).

IR (KBr) cm$^{-1}$: 2600-3304, (OH,NH,NH$_2$), 1776, (β-lactam, -C=O), 1714-1722 (Amide, -C=O), 1612, (Carboxylic, -C=O)

Preparation of 7-amino-3-[(Z/E)-propenyl]-3-cephem-4-carboxylic acid (16)

4-Methoxybenzyl 7-phenylacetamido-3-[(Z/E)-propenyl]-3-cephem-4-carboxylate 25 (50 g, 0.0954 mol) was dissolved in dichloromethane (500 ml), trimethylsilyl chloride (5 ml) was added at 0°C, stirred for 10 min. The reaction mass is cooled to -55°C, N, N-dimethylaniline (29.11 g, 0.241 mol) was added at -50 to -55°C and stirred for 10 min. PCl$_5$ (41.4 g, 0.199 mol) was added at -40 to -55°C and stirred at -35 to -40°C for 4 hrs. Methanol (100 ml) was added in 45-50 min at -35 to -20°C and stirred for 3 hrs. A 20 % w/v aq. NaCl solution (400 ml) was added and stirred for 15 min at 10°C. The layers were separated, aqueous layer was extracted with dichloromethane (100 ml) and the combined organic layer was concentrated to one
fifth of its original volume. Trifluoroacetic acid (50 ml) was added to the concentrated organic layer at 20 to 22°C in about 20-30 min and stirred for 8 to 10 hrs at 17 to 20°C. Water (400 ml) was added at 18 to 20°C and stirred for 20 min. After separating the layers, the organic layer is extracted with water (100 ml). Activated carbon (2 g), and EDTA (0.5 g) were added to the aqueous layer, stirred for 15 min at 20°C, filtered through celite and washed with water (100 ml). The pH of the filtrate was adjusted to 3.5 by adding 20% w/v aq. NaOH solution over a period of 60 min, the slurry is stirred, filtered and solid was washed with water (2 x 100 ml), acetone (2 x 100 ml); dried under vacuum at 40-45°C till the moisture content was not more than 0.5% w/w (yield 19 g; 76%; purity 97.39%, Z/E ratio 89/11 by HPLC).

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{CHOH} & \quad \text{CHOH} \\
\text{16}
\end{align*}
\]

\(^1\text{H NMR Spectrum (DMSO-\text{d}_6, \delta in ppm)}:
\]

1.61 d (3H, (E)-CH\text{CH}_3), 1.77 d (3H, (Z)-CH\text{CH}_3), 3.37-3.81 m (2 H, -S-CH\text{H}_2), 4.74 d (1 H, CO.CH-CH), 4.98 d (1 H, CO.CH-CH), 5.99—6.14 m (1H, -CH=CH(CH=CH\text{CH}_3)), 6.60—
6.68 d(1H, -CH=CH(CH=CH\text{CH}_3)).

IR (KBr) Cm\(^{-1}\): 3230,1805,1620, 1535

Theoretical mass :240

Mass : 239 (M-H)
Preparation of 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid (11)

Step A
To a mixture of methylene chloride (125 ml) and N,N-dimethyl formamide (85 ml), cooled to 20-25°C, was added a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml) and ethyl chloroformate in dichloromethane (12.72 g, 0.117 mole in 10 ml) under stirring. The resulting solution was cooled to −40° to −50°C and potassium D-N-(2-methoxycarbonyl-1-methylvinyl)-α-amino-α-(4-hydroxyphenyl) acetate (33.14 g, 0.11 mol) was added to it. The suspension is agitated at −40° to −35°C for 120 minutes. The reaction mass which was a solution of mixed anhydride product was cooled to −70 °C for condensation.

Step B
7-APCA (25 g, 0.104 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (9.3 g, 0.086 mole) and hexamethyl disilazane (13.4 g, 0.083 mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silylated 7-amino-3-(propan-1-yl)-3-cephem-4-carboxylic acid (7-APCA) compound.

Step C
To a solution of the mixed anhydride product of procedure 1A, cooled to −70 °C, was added with stirring, a cooled solution of the disilylated 7-APCA as prepared by procedure 1B. The reaction mixture was stirred at −50° to −40°C and monitored by
HPLC till quantitative conversion to the silylated Cefprozil was achieved. The reaction time was about 4 hours. The resulting reaction mass was added to a mixture of 85 ml water and 20 ml concentrated hydrochloric acid maintained at $-40^\circ$ to $-20^\circ$C. The temperature of the reaction mass was raised to 5 to $10^\circ$C and the pH of the solution was adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass was stirred for 30 minutes and the layers are separated. The aqueous was diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature was maintained at 10 to $15^\circ$C.

The aqueous solution containing Cefprozil was added to prechilled DMF (300 ml) at 15-18°C. The reaction mixture was basified to pH 6 to 6.5 by ammonia solution. The solid was stirred for 2.0 h at 20-25°C. The DMF solvate of Cefprozil was filtered off and washed with DMF (50 ml) followed by ethyl acetate (500 ml). The wet DMF solvate without drying is desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl acetate (45 ml) for 60 minutes. The product was filtered and dried to give Cefprozil monohydrate in the form of an off white to pale yellow crystalline powder. Yield: 30 gms, % Yield: 70.7, Purity: 101.2%, Total impurities: 0.45%

![Chemical Structure](image-url)
$^1$H NMR Spectrum (DMSO-d$_6$, $\delta$ in ppm):

1.61–1.64 d (3H, (Z)-CH$_3$), 1.69–1.72 d (3H, (E)-CH$_3$), 3.26–3.57 m (2H, -S-CH$_2$),
4.71 s (1H, -CO-CH-NH$_2$), 4.91–d (1H, CO-CH-CH) 4.93 d (1H, CO-CH-CH),
5.37–5.46 m (1H, -CH=CH(CH$_3$)), 6.23–6.28 d (1H, -CH=CH(CH$_3$)), 6.66–6.75
m(2H, benzene-H Ortho to –CH-NH$_2$), 7.17–7.25 m (2H, benzene-H para to CH-
NH$_2$), 8.84–8.94 m (2H, -NH$_2$).

IR (KBr) cm$^{-1}$: 3546, 1758, 1683, 1563, 1515, 1461, 1396, 1343, 1312, 1269, 1234.

Theoretical mass: 389

Mass: 390 (M+H), 407 (M+H), 412 (M+H)

Preparation of 7-[D-$\alpha$-amino-$\alpha$-(4-hydroxyphenyl) acetamido]-3-(1-
propen-1-yl)-3-cephem-4-carboxylic acid (11)

Step A

Methylene chloride (125 ml) was cooled to 20-25°C, and a solution of N-
methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml) and ethyl
chloroformate in dichloromethane (12.72 g, 0.117 mole in 10 ml) were added under
stirring. The resulting solution was cooled to $-$40° to $-$50°C and potassium D-N-(2-
methoxycarbonyl-1-methylvinyl)-$\alpha$-amino-$\alpha$-(4-hydroxylphenyl) acetate (33.14 g,
0.11 mol) is added to it. The suspension is agitated at $-$40° to $-$35°C for 90 minutes.
N,N-dimethyl formamide (85 ml), cooled to $-$70°C, was added and the suspension
was further agitated for 30 minutes. The reaction mass which was a solution of
mixed anhydride product was cooled to $-$70 °C for condensation.
Step B

7-APCA (25 g, 0.104 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (9.3 g, 0.086 mole) and hexamethyldisilazane (13.4 g, 0.083 mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silylated 7-aminocarbonyl-3-(propan-1-yl)-3-cephem-4-carboxylic acid (7-APCA) compound.

Step C

To a solution of the mixed anhydride product of procedure 2A, cooled to −70 °C, was added with stirring, a cooled solution of the disilylated 7-APCA as prepared by procedure 2B. The reaction mixture was stirred at −50° to −40°C and monitored by HPLC till quantitative conversion to the silylated Cefprozil was achieved. The reaction time was about 4 hours. The resulting reaction mass was added to a mixture of 85 ml water and 20 ml concentrated hydrochloric acid maintained at −40° to −20°C. The temperature of the reaction mass was raised to 5° to 10°C and the pH of the solution was adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass was stirred for 30 minutes and the layers are separated. The aqueous layer was diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature was maintained at 10° to 15°C.

The aqueous solution containing Cefprozil as obtained above was converted to its DMF solvate as per example (3). The wet DMF solvate without drying was desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl
acetate (45 ml) for 60 minutes. The product was filtered and dried to give Cefprozil monohydrate in the form of an off white to pale yellow crystalline powder.

Yield: 33.4 gms, % Yield: 78.7, Purity: 101.3%, Total impurities: 0.5%

\[ \text{\includegraphics{image}} \]

\(^{1}\)H NMR Spectrum (DMSO-d\(_6\), \(\delta\) in ppm):

1.61–1.64 \(d\) (3H, (Z)-CH\(_3\)), 1.69–1.72 \(d\) (3H, (E)-CH\(_3\)), 3.26–3.57 \(m\) (2H, -S-CH\(_2\)), 4.71 \(s\) (1H, -CO-CH-NH\(_2\)), 4.91–d (1H, CO-CH=CH) 4.93 \(d\) (1H, CO-CH:CH), 5.37–5.46 \(m\) (1H, -CH=CH(CH\(_3\)) ), 6.23–6.28 \(d\) (1H, -CH=CH(CH\(_3\))), 6.66–6.75 \(m\) (2H, benzene-H Ortho to -CH-NH\(_2\)), 7.17–7.25 \(m\) (2H, benzene-H para to CH-NH\(_2\)), 8.84–8.94 \(m\) (2H,-NH\(_2\)).

IR (KBr) \(\text{Cm}^{-1}\): 3546,1758,1683,1563,1515,1461,1396,1343,1312 1269,1234.

**Theoretical mass**: 389

**Mass**: 390 (M+H), 407 (M+H), 412 (M+H)

**Example –5**

(6R)-3-((1Z)prop-1-enyl)-6-[2-amino-2-(4-ethoxycarbonyloxyphenyl)acetylamino]–5-oxo-6aH-azetidino[2,1-b]1,3-thiazine-4-carboxylic acid .(Carbonate impurity)

Step A
Methylene chloride (125 ml) was cooled to 20-25°C, and a solution of N-methylmorpholine in dichloromethane (10.52 g, 0.104 mole in 15 ml) and ethyl chloroformate in dichloromethane (24.86 g, 0.229 mole in 10 ml) were added under stirring. The resulting solution was cooled to −40° to −50°C and potassium D-N-(2-methoxycarbonyl-1-methylvinyl)-α-amino-α-(4-hydroxylphenyl) acetate (33.14 g, 0.11 mol) was added to it. The suspension was agitated at −40° to −35°C for 90 minutes. N,N-dimethyl formamide (85 ml), cooled to −70°C, was added and the suspension was further agitated for 30 minutes. The reaction mass which was a solution of mixed anhydride product is cooled to −70 °C for condensation.

Step B

7-APCA (25 g, 0.104 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (9.3 g, 0.086 mole) and hexamethyldisilazane (13.4 g, 0.083 mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silylated 7-aminocarbonyl-3-(propan-1-y)-3-cephem-4-carboxylic acid (7-APCA) compound.

Step C

To a solution of the mixed anhydride product of procedure 2A, cooled to −70 °C, was added with stirring, a cooled solution of the disilylated 7-APCA as prepared by procedure 2B. The reaction was stirred at −50° to −40°C and monitored by HPLC till quantitative conversion to the silylated Cefprozil was achieved. The reaction time was about 4 hours. The resulting reaction mass was added to a mixture of 85 ml water and 20 ml concentrated hydrochloric acid maintained at −40° to −20°C. The
temperature of the reaction mass was raised to 5° to 10°C and the pH of the solution was adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass was stirred for 30 minutes and the layers are separated. The aqueous layer was diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature was maintained at 10° to 15°C.

The aqueous solution containing impurity rich Cefprozil as obtained above was converted to its DMF solvate as per example(3). The wet DMF solvate without drying was desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl acetate (45 ml) for 60 minutes. The product was filtered was purified by the column chromatography using the resin XAD –1180 at pH 6.0-6.5. The elute then acidified to pH 2.8 to 3.2 by 10% hydrochloric acid solid formation takes place. The solid was filtered off and dried under vacuum, 16.0 gm of title impurity was collected in the pure form.

Molecular formula: C_{21}H_{23}O_{7}N_{3}S

Molecular weight: 461.4

Mass: 462.4 (M+H), 479.2 (M+H), 484 (M+H)

^1H NMR Spectrum (DMSO-d_6, δ in ppm):
1.25-1.32 t (3H, -OCH₂-CH₃), 1.62-1.66 d (3H, -CH=CH(CH₃)), 3.51-3.56 dd (2H, -S-CH₂), 4.19-4.26 q (2H, -OCH₂CH₃), 4.46 d (1H, CO-CH-CH), 4.55 d (1H, CO-CH-CH), 5.1 s (1H, -CO-CH-NH₂), 5.32-5.41 m (1H, -CH=CH(CH₃)), 5.71-5.75 d (1H, -CH=CH(CH₃)), 7.21-7.25 m (2H, benzene-H Ortho to -CH-NH₂), 7.37-7.41 m (2H, benzene-H Para to CH-NH₂), 8.84-8.94 d (2H, -NH₂).

**Example – 6**

3-((1Z)prop-1-enyl)-6-{2-[2-amino-2-(4-hydroxyphenyl)acetylamino]-2-(4-hydroxyphenyl)acetylamino}-5-oxo-2H,6H.6aH-azetidino[2,1-b]1,3-thiazine-4-carboxylic acid (N-Glycil impurity).

**Step A**

Methylene chloride (125 ml) was cooled to 20-25°C, and a solution of N-methylmorpholine in dichloromethane (0.25 g, 0.0025 mole in 5 ml) and ethyl chloroformate in dichloromethane (7.47 g, 0.0688 mole in 10 ml) were added under stirring. The resulting was cooled to –40° to –50°C and potassium D-N-(2-methoxycarbonyl-1-methylvinyl)-α-amino-α-(4-hydroxyphenyl) acetate (20.07 g, 0.0662 mol) was added to it. The suspension was agitated at –40° to –35°C for 90 minutes. N,N-dimethyl formamide (85 ml), cooled to –70°C, was added and the suspension was further agitated for 30 minutes. The reaction mass which was a solution of mixed anhydride product was cooled to –70°C for condensation.
Step B

Cefprozil (25 g, 0.0614 mole) was added to methylene chloride (100 ml) at 25° to 30°C followed by addition of N,O-bis-trimethylsilyl acetamide (0.125 g, 0.0006 mole), trimethylchlorosilane (10.94 g, 0.100 mole) and hexamethyl disilazane (15.81 g, 0.098 mole). The reaction mass was heated to reflux temperature and refluxed for 4 hours to obtain silylated 7-[D-α-amino-α-(4-hydroxyphenyl) acetamido]-3-(1-propen-1-yl)-3-cephem-4-carboxylic acid (Cefprozil) compound.

Step C

To a solution of the mixed anhydride product of procedure 2A, cooled to −70 °C, was added with stirring, a cooled solution of the disilylated Cefprozil as prepared by procedure 2B. The reaction mixture was stirred at −50° to −40°C and monitored by HPLC till quantitative conversion to the silylated Cefprozil was achieved. The reaction time was about 4 hours. The resulting reaction mass was added to a mixture of 85 ml water and 20 ml concentrated hydrochloric acid maintained at −40° to −20°C. The temperature of the reaction mass was raised to 5° to 10°C and the pH of the solution is adjusted to 0.5 with concentrated hydrochloric acid. The reaction mass was stirred for 30 minutes and the layers are separated. The aqueous layer was diluted with acetone (75 ml) followed by the addition of 2.5 g of activated carbon and sodium dithionite (0.25 g) to remove the colored solid impurities. The reaction temperature was maintained at 10° to 15°C.

The aqueous solution containing N-glycl Cefprozil as obtained above was converted to its DMF solvate as per the method given in example (3). The wet DMF solvate of
N-glycyl Cefprozil without drying was desolvated by stirring with a mixture of demineralised water (75 ml) and ethyl acetate (45 ml) for 60 minutes. The product was filtered and dried to give title impurity (27) in the form of an off white to crystalline powder.

Yield: 20 gm.

Molecular formula: $C_{28}H_{28}O_7N_4S$

Molecular weight: 538.4

Mass: 539.4(M+H), 561.3 (M+H), 562.2 (M+H)

$^1$H NMR Spectrum (DMSO-$d_6$, $\delta$ in ppm):

1.66 d (3H, -CH=CH (CH$_3$)), 3.28-3.59 m (2H, -S-CH$_2$), 4.71 d (1 H, CO-CH-CH), 4.55 d (1 H, CO-CH-CH), 4.94 s (1H, -CO-CH-NH$_2$), 5.34—5.55 m (1H, -CH=CH(CH$_3$), 6.25—6.31 d (1H, -CH=CH(CH$_3$)), 6.67—6.77 m (4H, benzene-H Ortho to -CH-NH$_2$), 7.18—7.26 m (4H, benzene-H Para to CH-NH$_2$), 8.85—8.96 d (2H,-NH$_2$).
Cefprozil Carbonate (26)
3.7 References:

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