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Research Article

PROCESS DEVELOPMENT FOR SYNTHESIZING CEFETAMET SODIUM

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ABSTRACT

The research work relates to an improved and cost effective process for the industrial manufacture of Cefetamet Sodium. 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. It can be possible with by using intermediate MAEM to synthesize Cefetamet Sodium. The drug is registered in USP and belongs to 3rd generation drug. There are many patents which gives the procedure of Synthesis of Cefetamet Sodium. These synthesis procedures have taken as standard procedure for pursuing the project work. At present, MAEM is highly used intermediate to synthesis cephalosporin antibiotics. In this project work modification has done for synthesizing of cephalosporin antibiotics by utilizing MAEM as intermediate and other chemicals like different Lewis acid, and solvent as alternate of intermediate, chemicals and solvents which are given in patents to synthesize cephalosporin antibiotics .Resulting yield has improved via making small time synthesis reaction .This is helpful for the commercial purpose. Cephalosporins are the highly used Broad spectrum antibiotics; belong to β-lactam class. The bactericidal action of beta lactam antibiotics is directly attributable to their ability to react with PBP's. The Reaction monitoring was done by HPLC and identification of final product was done by MASS, I.R, NMR and then comparing with the well known literature. Recovery of the side product and byproduct (mercaptobenzothiazole) were achieved to allow a greener process and utilizes for synthesis of other familiar drugs.

Keywords: Cefetamet Sodium, IR, NMR, TLC, HPLC, Mercaptobenzothiazole

1. INTRODUCTION:

Since some years ago the synthesis of antibiotics is a branch of pharmaceutical chemistry to which some investments have been made in order to develop new compounds with antimicrobial activity. In 1945, Brotzu discovered the fungus *Cephalosporium Acremonium* which produce a chemical which show antimicrobial activity. In 1948 Abraham at the Sir William Dunn School of Pathology at the University of Oxford and his colleagues have been supplied cultures of the fungus and were isolated three principal antibiotic components :i) Cephalosporin P, (a steroid antibiotic that resembles fusidic acid) with minimal antibacterial activity. ii) Cephalosporin N, later discovered to be identical with synnematin N (a penicillin derivative now called penicillin N.iii) Cephalosporin C it was isolated in 1952 from a mold of the genus Cephalosporium.[1]

A decade later the nucleus (7-aminocephalo-sporanic acid) was isolated and used as the basis for a series of synthetic derivatives, including Cephalothin, cephaloridine and Cephaloglycin. (a cephem containing ring now called nucleus of cephalosporin drugs). He noticed that these cultures produced substances that were effective against *Salmonella Typhi*, the cause of typhoid fever, which had beta-lactamase. The cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA), was derived from cephalosporin C and proved to be analogous to the penicillin nucleus 6-aminopenicillanic acid, but it was not sufficiently potent for clinical use. Modification of the 7-ACA side-chains resulted in the development of useful antibiotic agents, and the first agent is Cephalothin (cefalotin) was launched by. Eli Lilly Company in 1964.

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The Cephalosporins are a class of β-lactam antibiotics originally derived from Acremonium, which was previously known as "Cephalosporium". Together with cephamycins they constitute a subgroup of β-lactam antibiotics called cephem.[2,3] Cephalosporins are among the most important antibiotics. Cephalosporins resemble penicillin in that they have a β-lactam structure, but the five-member thiazolidine ring characteristic of the penicillin is replaced by a six-member dihydrothiazine ring .The dihydrothiazine ring of the cephalosporin provides the molecule with the ability to resist bacterial enzymes; the antibacterial activity emanates from the β-lactam ring shared by penicillin and Cephalosporins.[4]

Modifications at position 7 of the cephalosporin nucleus generally affect the antibacterial spectrum, and substitutions at position 3 of the dihydrothiazine ring alter the pharmacokinetics and metabolic parameters of the drug. In an effort to obtain derivatives possessing a broader antibacterial spectrum, greater stability towards lactamases and improved pharmacological properties, modifications of the cephem basic skeleton is required. In cephalosporin nucleus there are two positions available for chemical manipulation, C3 and C7. A wide variety of amine acylation methods have been used for the production of C7- acylamino derivatives by the use of acyl chlorides, mixed anhydrides, active esters, and carbodiimides to improve pharmacodynamic property . To improve the chemical reactivity of 7-ACA, the solubility in organic solvents is increased by conversion of the carboxylic acid at C4 of 7-ACA into an ester such as tert-butyl dimethylsilyl, benzhydry1,p-nitrobenzyl, o-p-methoxybenzyl to improve pharmacokinetic property .

The cephalosporin nucleus can be modified to gain different properties. Cephalosporins are sometimes grouped into "generations" by their antimicrobial properties. The first Cephalosporins were designated first-generation Cephalosporins, whereas, later, more extended-spectrum Cephalosporins were classified as second-generation Cephalosporins. Each newer generation of Cephalosporins has significantly greater Gram-negative antimicrobial properties than the preceding generation, in most cases with decreased activity against Gram-positive organisms. Fourth-generation Cephalosporins, however, have true broad-spectrum activity.[5]

2. MATERIALS & METHODS:

- The laboratory grade reagents and chemicals were used to synthesize title compounds.
- The 1H- NMR Spectra were recorded on a BRUKER ADVANCE II 400. Spectrometer using TMS as internal standard, DMSO as a solvent.
- MS were recorded in a By Mass Spectroscopy :Agilent Triple Quad System LC-MS-MS System 6410) based on the electronic impact technique with EI=70 eV and DMK 400 V. pH measurements were carried out in aqueous solution on 10% w/v at 25°C in a Crison microp H 2001.
- The IR spectra were 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).

Experimental Part

2.1 Art of synthesis of cefetamet sodium

This antibiotic is obtained from 7-ADCA by single step synthesis. Its structure is characterized by the presence of a methyl group linked to position three of the cephalosporanic nucleus & carboxylic group at 4th position. These are characteristics associate to its highly parenteral absorption. Moreover, the 2-aminothiazole ring linked to position 7 by means of a (methoxyimino) acetamido system gives a high antimicrobial potency against Gram (-) germs and high resistance against -lactamases. In this case, it is necessary to built two fundamental molecular fragments which should be coupled each other and together, so several strategies of synthesis have been made in order to obtain them.[6]

Its structural characteristic as shown in Figure:

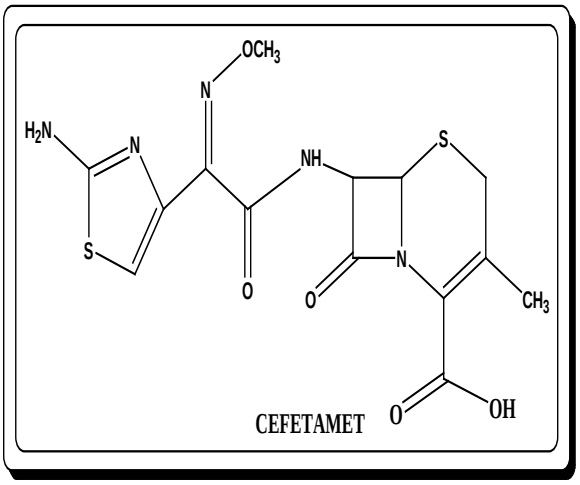


Figure 1: Structure of cefetamet

2.2 Principal Reaction Scheme to Synthesize Cefetamet Acid

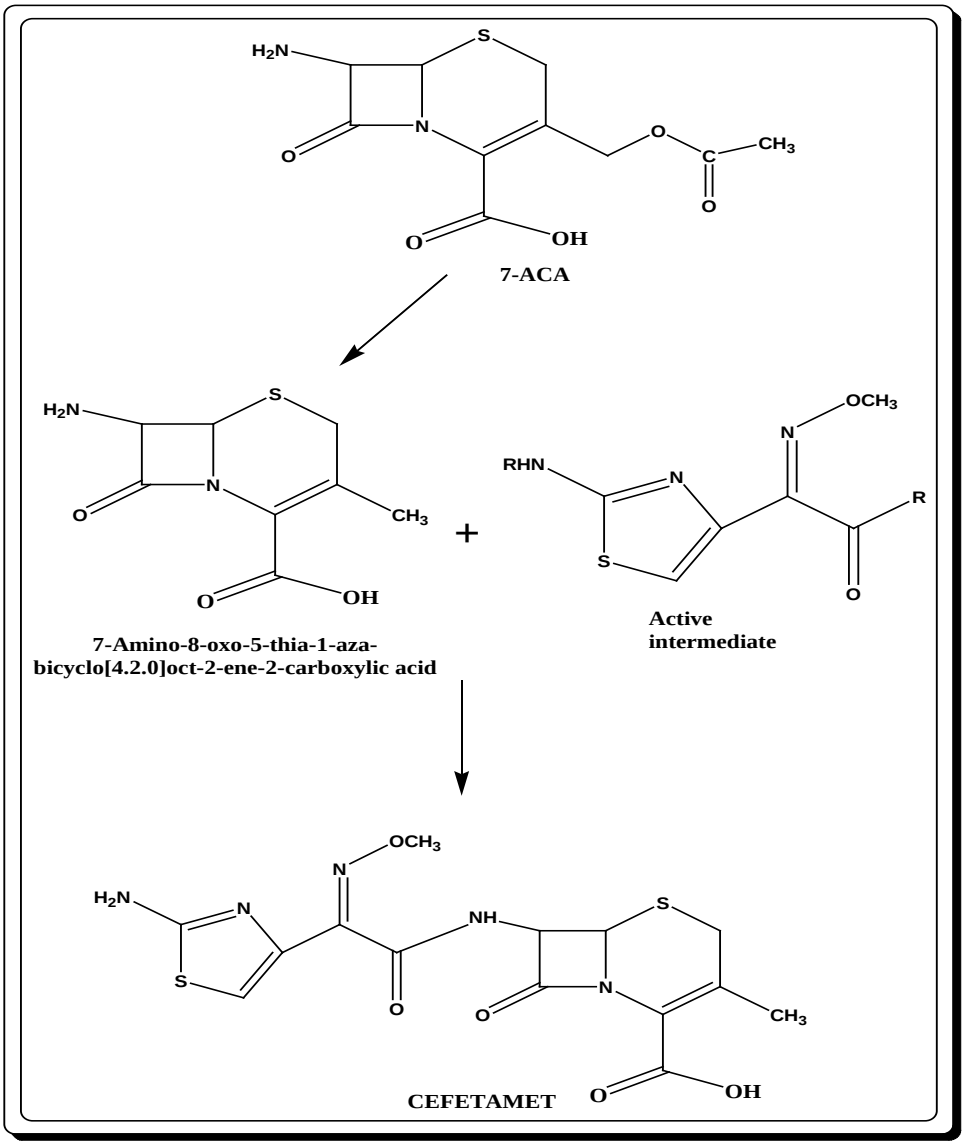


Figure 2: Scheme to Synthesize Cefetamet Acid

2.3 General Principal reaction for synthesizing Cefetamet Sodium

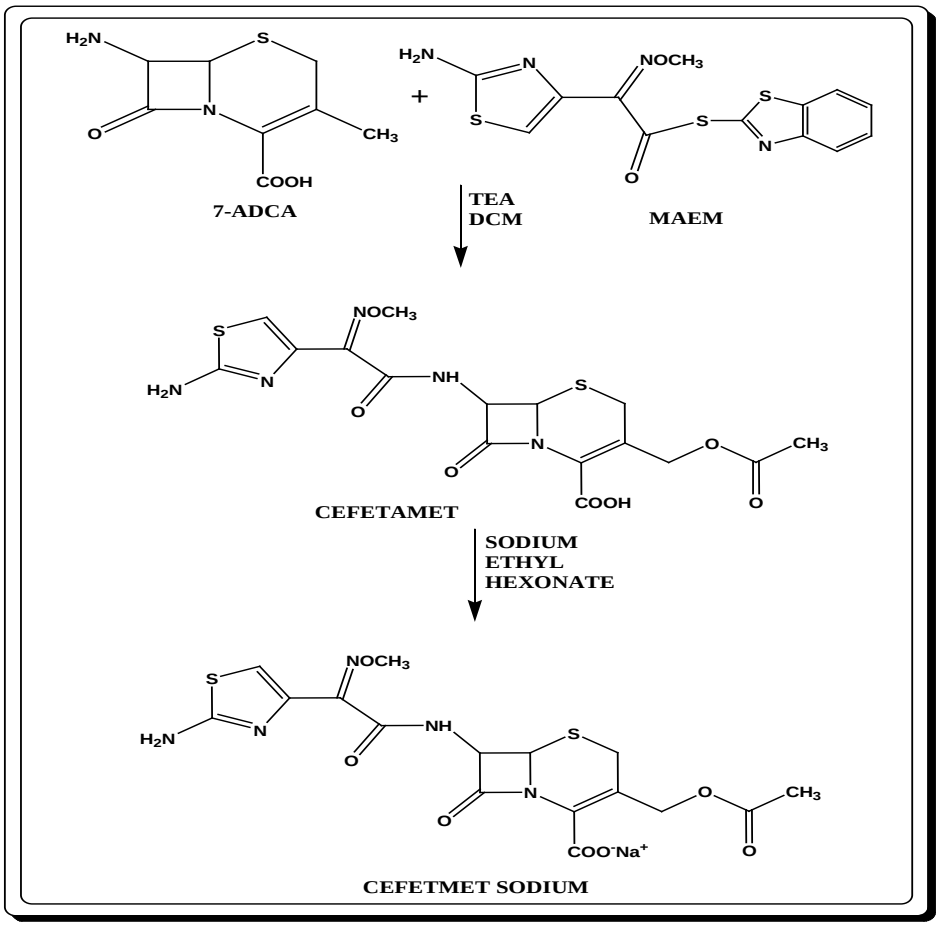
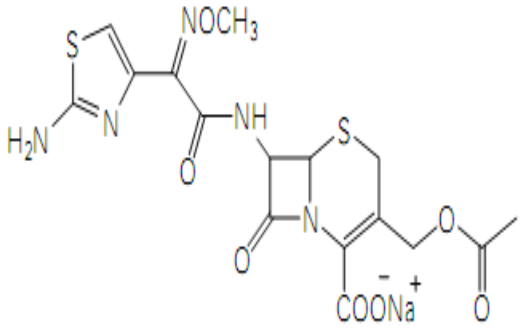


Figure 3: Principal reaction for synthesizing Cefetamet Sodium

2.4 Synthetic procedures for preparaing cefetamet sodium

Preparation of cefetamet sodium salt

Sodium 3-methyl-7-[2-(2-amino-thiazol-4-yl)-2-methoxyimino-acetylamino]- 8-oxo-5-thia-1-aza-bicyclo [4.2.0] oct-2-ene-2-carboxylate (2)



- Charged Methanol 110 ml and wet 7 – AMCA at RT
- Cool down to 10^0c , charged MAEM 23 gm at 10^0c and flush with 10 m MeOH.
- Added TEA 7.5 gm slowly In 30 to 45 min. at 10 – 12^0c
- Stirred reaction mix at 10-12^0c till reaction completion.
- After reaction completion charged 560 ml DM water and adjusted pH 5.4 to 5.5 with 5% sulphuric acid.
- Cooled down to 5^0c and stirred 20 min.
- Filtered and washed MBT with 50 ml DM water.
- Took clear filtrate and charged 2.5 gm carbon , 0.5 gm hydro and 0.25 gm EDTA.
- Stirred 30 min. at 20 -25^0c.
- Filtered and washed carbon bed with 50 ml DM water.
- Took clear filterate and added 5% sulphuric acid up to haziness at 25 – 30^0c.

- Stirred 30 min. at 25 – 30^{0}c.
- Finally adjusted pH 2.5 with 5% sulphuric acid.
- Cooled down to 0 – 5^{0}c. and stirred 60 min at 0 -5^{0}c.
- Filtered and washed with –(a) 50 ml DM water
- (b) 2*90 ml Acetone.
- Sucked properly and unloaded the wet material.
- Dried in the TD at 45 to 50^{0}c till moisture content less than 1.2% w/w.
- To a suspension of 50.0 g (110 mmol) of 1 in a mixture of 110 mL of water and 90 mL of ethanol was added 8.78 g (105 mmol) sodium ethyl hexonate suspended in 25 mL of ethanol.
- The resulting solution was treated with 5 g of activated charcoal and stirred during 15 min.
- The mixture was vacuum filtered, the residue was washed successively with 250 mL of ethanol and 100 mL of water and filtrates were combined and evaporated to dryness.
- The residue was dissolved in 110 mL of methanol and poured into 2.2 L of diethyl ether under stirring.
- The precipitate was filtered, washed with diethyl ether (2 x 50 mL) and vacuum dried during 3-4 hr at 35-40 °C affording 50 g (90.4% yield) of 2.
- The residual organic layer obtained, after extraction of cefetamet with water, was washed with 140 mL of 2 M NaOH solution
- The aqueous layer was then acidified with 43 mL of 6 M hydrochloric acid. The precipitate was filtered, washed with water (3 x 50 mL) and dried during 1 h at 100°C.

3. RESULTS AND DISCUSSION:

3.1. Physical Parameters[7, 8]

The Physical appearances and odors of following compound are:

Table: 1

Compound	Appearances	Color	Odors
Cefetamet sodium	Coarse Powder	Whitish to yellowish brown	odorless

Table 1.1: List of Chemicals Used In Isolation Process

Chemical Name	Grade	Company
ACETONE	B (For Synthesis)	SPECTROCHEM
TEA	A (For Synthesis)	Qualigens Fine Chemicals, Mumbai
BSA	A (For Synthesis)	ALDRICH
ETHYL ACETATE	A (For Synthesis)	SPECTROCHEM
SULFOLANE	A (For Synthesis)	S.D Fine Chemicals, Mumbai
METHANOL	B (For Synthesis)	SPECTROCHEM
7ACA		SANDOZ
SULPHURIC ACID	B (For Synthesis)	SPECTROCHEM
MDC	B (For Synthesis)	SPECTROCHEM
THF	B (For Synthesis)	SPECTROCHEM
EDTA	B (For Synthesis)	SPECTROCHEM
MAEM		SANDOZ
CARBON	A	SPECTROCHEM
SODIUM IODIDE	A (For Synthesis)	MERCK
POTASSIUM BROMIDE	B (For Synthesis)	MERCK
SODIUM THIOSULPHATE	B (For Synthesis)	ALDRICH
TOLUENE	A (For Synthesis)	SPECTROCHEM
CROWN ETHER	A (For Synthesis)	ALDRICH
CHLORO COMPD.	B (For Synthesis)	SPECTROCHEM

SODIUM SULPHATE	B (For Synthesis)	SPECTROCHEM
AMMONIA	B (For Synthesis)	SPECTROCHEM
SODIUM HYDROXIDE	B (For Synthesis)	Qualigens Fine Chemicals, Mumbai
DMAC	B (For Synthesis)	SPECTROCHEM
Hydrochloric Acid		Central Drug House (p)ltd. New Delhi
Ortho-phosphoric acid	A (For Synthesis)	ALDRICH
7-ADCA	A (For Synthesis)	SANDOZ
Sodium ethyl Hexonate	B (For Synthesis)	Central Drug House (p)ltd. New Delhi
Dimethyl formamide	A (For Synthesis)	SPECTROCHEM

Table 1.2: List of Chemicals Used In TLC and HPLC analysis

Si. No.	Name of Chemicals	Grade of chemicals
1	Chloroform	HPLC grade, Merck
2	Toluene	AR grade, Merck
3	Methanol	HPLC grade, Merck
4	Acetonitrile	HPLC grade, Merck
5	Formic acid	AR grade, Merck
6	Glacial acetic acid	AR grade, Merck
7	Acetone	HPLC grade, Merck
8	Ethyl acetate	AR grade, Merck
9	Water for HPLC	HPLC grade, Merck

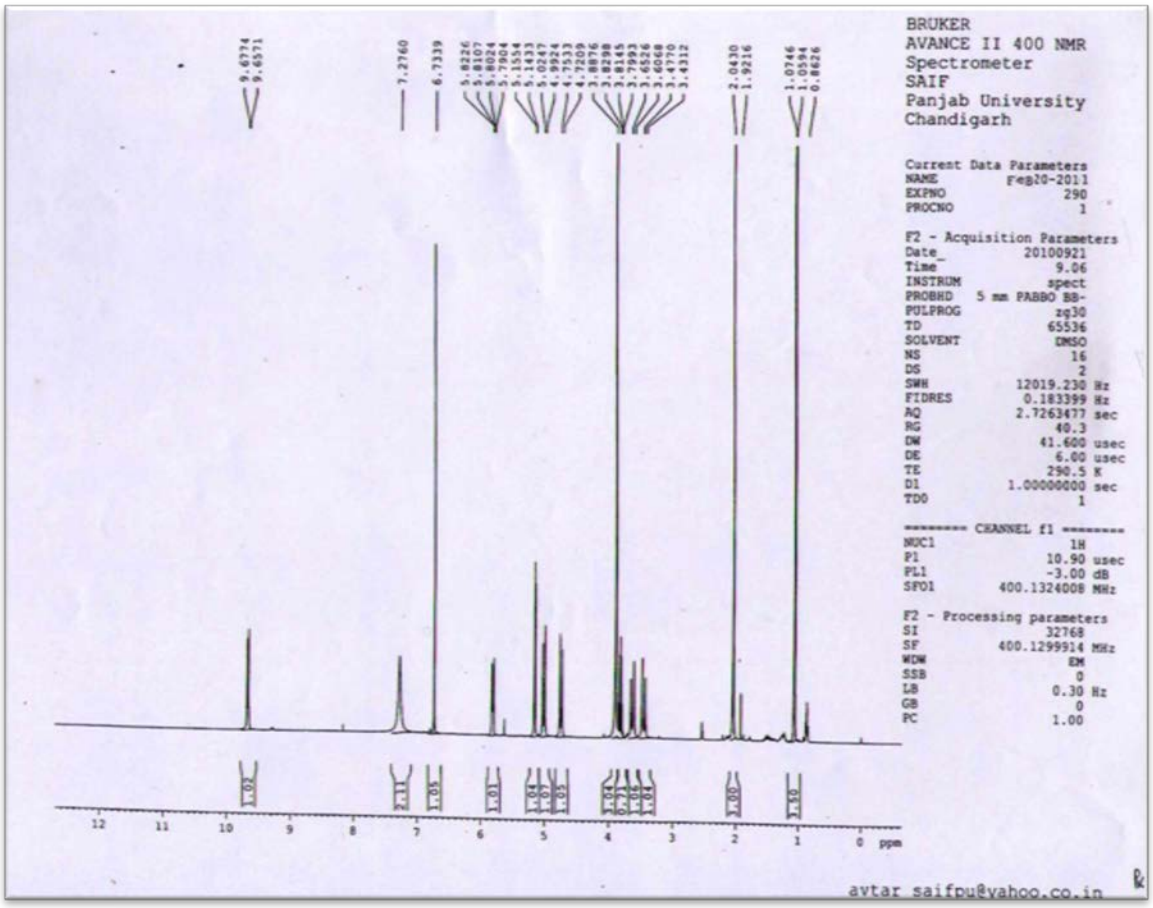
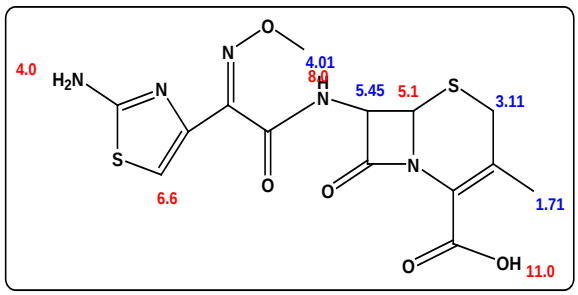


Figure 4: NMR Spectra of Cefetamet



Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH	5.45	3.08	propiolactam
		2.10	1 alpha -N-C=O from methine
		0.27	1 beta -SR from methine
		?	1 unknown substituent(s)
CH	5.1	3.42	propiolactam
		1.04	1 alpha -SR from methine
		0.62	1 beta -N-C=O from methine
		?	1 unknown substituent(s)
		?	1 -R from N-CHx
			-> 1 increment(s) not found
CH2	3.11	1.37	methylene
		0.63	1 alpha -C=C
		1.11	1 alpha -S-C
OH	11.0	11.00	carboxylic acid
NH	8.0	8.00	sec. amide
CH3	1.71	0.86	methyl
		0.85	1 alpha -C=C
CH	6.6	7.41	thiazole
		-0.85	1 -N from 2-thiophene
		?	1 unknown substituent(s) from 3-thiophene
		?	1 unknown substituent(s) from 3-furan
			-> 1 increment(s) not found
NH2	4.0	4.00	aromatic C-NH
CH3	4.01	0.86	methyl
		3.15	1 alpha -O-N=C

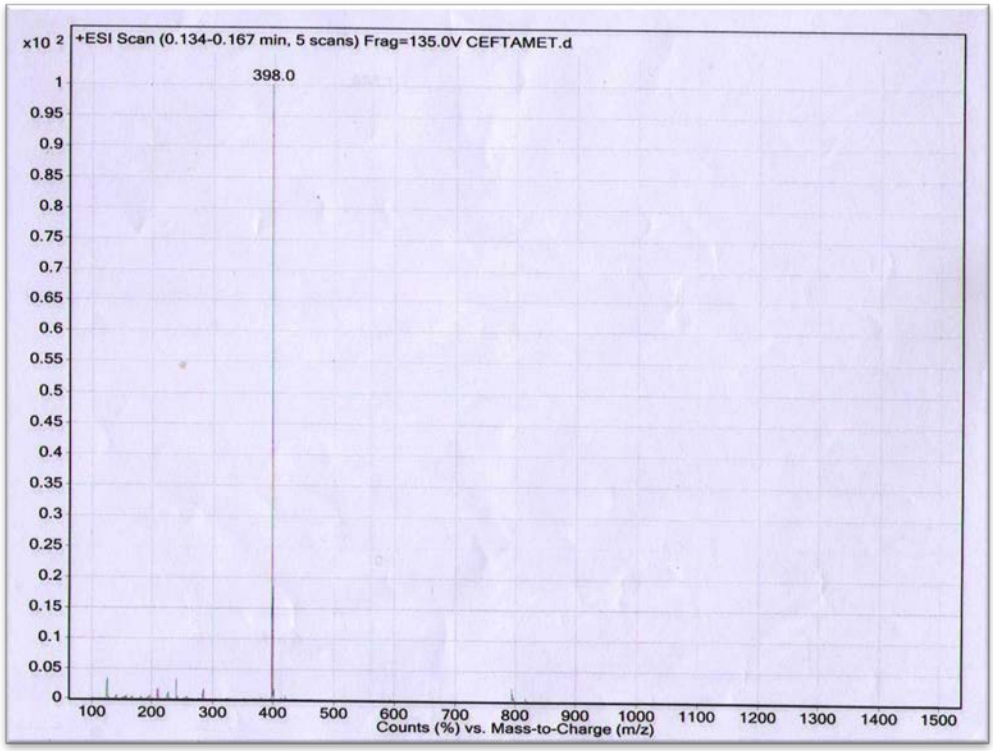


Figure 5: Mass Spectra of Cefetamet Sodium

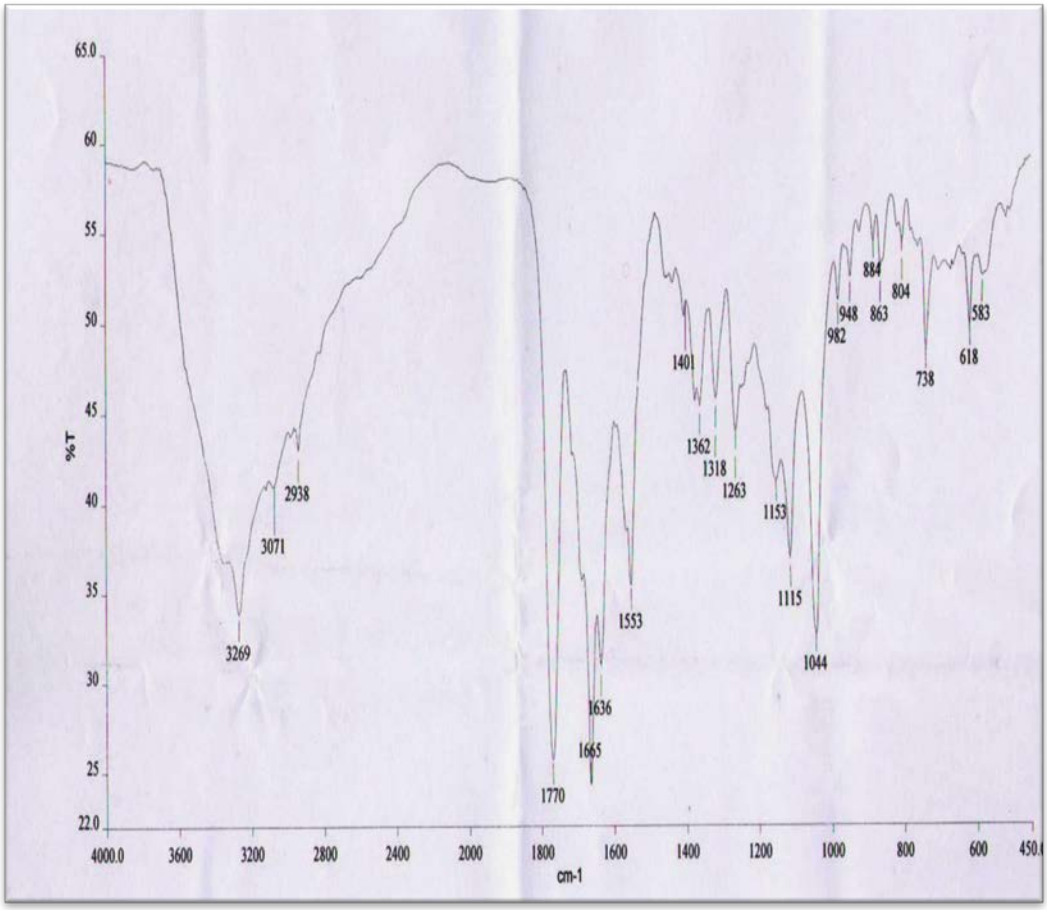


Figure 6: I.R. Spectra of Cefetamet

3.2 Solubility Parameters[9, 10]

The solubility of synthesized compounds is as follows:

Table: 2

Compound	Solubility
Cefetamet Sodium	Freely soluble in water, sparingly Soluble in methanol &very slightly soluble in alcohol.

3.3 Determination of Melting Point Range:

Melting points of the newly synthesized compounds were determined by open capillary method using the melting point apparatus. The melting points of synthesized compounds are given in

Table 3:

Compound	Melting point (^{0}c)
Cefetamet Sodium	125-128

3.4 HPLC Parameters of Compounds [11, 12]

The retention times of following compounds are as follows:

Table: 4

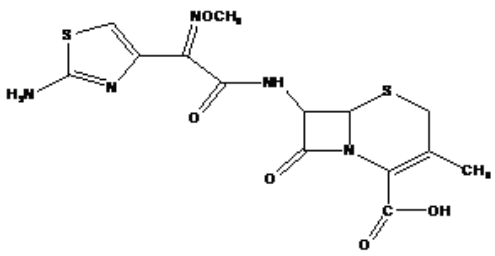
s.no	Compounds	Retention Time
1	7-ACA	6.26-6.30
2	AMCA	3.80-3.90
3	ADCA	3.23-3.27
4	Chloro Compd.	8.51-8.55
5	Iodo Compd.	10.50-10.55
6	MAEM	16.70-16.75
7	CPDA	7.92-7.83
8	CPPN-A CPPN-B	10.78-10.80 9.73-9.80
9	Cefetamet Sodium	6.24-6.26

Spectra Analysis

3.5 I.R of the synthesized compound:

The functional groups of synthesized compounds can be concluded from the value of I.R spectra, it is also an identification source of the compound:

Table: 5

COMPOUND	Common Value	Interpretation
	-1745 stretch 3400-3200 .1730-1700 -1690-1640 3350&3180 1740-1715	-β-lactam amide OH strof COOH C=O of acid -Oxime peak -Primary amine -Ester peak

3.6 Interpretations of mass spectra of follwing synthesizes compound:

Table: 6

Compound	Molecular Weight	Base Peak	Molecular ion peak
Cefetamet Sodium	397.43	398.0	398.0

3.7 Interpretations of nmr spectra of follwing synthesizes compound:

- CEFETAMET SODIUM:
- H-NMR (DMSO-d6): 9.64 (m, 1H, NHCO), 7.24 (s, 2H, NH2), 6.75 (s, 1H, thiazole ring), 5.85 (dd, 1H, H-7), 5.16 (d, 1H, H-6), 3.83 (s, 3H, NOCH3), 4.23(s, 3H,CH3).

These all described data confirms the synthesized drugs.

3.8 Yield of following synthesized products

The theoretical yield & practical yield are calculated on the basis of substrate i.e In case of CPPN and , 7-ACA is taken as substrate and &7-ADCA is taken as substrate for Cefetamet. All value of yield are calculates in reference of 50 g substrate.

Table: 7

Compound	Theoretical yield	Practical yield	%yield
Cefetamet Sodium	92.97gm	87.48gm	94.10

CONCLUSION:

The use of MAEM as starting material for preparing Cefetamet Sodium, allowed to obtain better yields for the synthesis of these antibiotics. Previous esterification of the chloroacetylated derivative followed by cleavage of the chloroacetyl protective group, allowed to eliminate the drawbacks of the classic pathways of synthesis, especially the final purification of Cefetamet Sodium by column chromatography. Moreover, the utilization of MAEM allows diminish the production cost of the final product. The reaction time has been reduced by lowering the time for acylation. The overall yield has been increased to 91% with good. Recovery of the byproduct (mercaptobenzothiazole) was achieved to allow a greener process.

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