ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis and Characterization of Cefotaxime Acid

Susi Kusumaningrum*, Alfan Danny Arbianto, Maya Damayanti Rahayu, Pertamawati, Eriawan Rismana, and Firdayani.

Center for Pharmaceutical and Medical Technology - LAPTIAB – BPPT, Puspiptek area, 611 Building – South Tangerang – Banten – Indonesia.

ABSTRACT

Cefotaxime is an antibiotic which has a high economic value as well as the necessary but expensive. The aim of this research is to find an efficient procedure for the synthesis of cefotaxime acid. The synthesis cefotaxime acid laboratory scale, has been carried out using the method with the number of catalysts, different acid and solvent. The synthesis of cefotaxime is reacting 7-aminoacephalosporanic acid with thioester reactant S-benzothiazol-2-yl(2-amino-4-thiazolyl) (methoxyimino)thioacetate. Synthesis product is characterized by using TLC, HPLC, 1HNMR, LCMS and FTIR. Physicochemical and microbiological testing is also done with the parameters purity, moisture content, ash content, residual levels of acetone, heavy metals contamination and microbial contamination. The results showed that the synthesis of cefotaxime using 1% TEA catalyst gives the highest yield of 86%. Analysis by FTIR, LC, MS and NMR showed that the cefotaxime synthesis product has similar characteristics to the standard of the EP cefotaxime. The antibacterial activity of cefotaxime demonstrated that both of the synthesis and standard cefotaxime able to inhibit the growth of *Escherichia coli ATCC25922, Salmonella typhimurium ATCC14028 and Bacillus subtilis* ATCC633 but there was no inhibition against *Staphylococcus aureus* ATCC25923. Cefotaxime synthesized having the same character with cefotaxime standards and met the requirements required.

Keywords: cefotaxime, Antibiotics, physical and chemical properties

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The availability of drugs in Indonesia, especially antibiotics are still few and dependent on imported products. The classic reason is the absence of the domestic industry is able to produce because of the unavailability of raw materials and production capabilities in mastering technology. Currently, the rate of dependence on imports of raw materials for drugs in Indonesia is still very high (95%) and is equivalent to 11.4 Trillion Rupiahs in 2012 [1]. The amount may be reduced if the raw materials of drugs can be produced locally. Mastery of development and production technology of drug raw materials is expected to play a role in the achievement of the Economic Package XI in the field of Pharmaceutical and Medical Devices and Presidential Instruction No. 6 of 2016 dated June 6, 2016 on accelerating the development of the pharmaceutical industry and medical devices [2].

Cefotaxime is necessary to meet the needs of antibiotics in the country is very large and has wide clinical applications for treatment of infections of the respiratory tract, gynaecologic, skin, bone and joint, urinary tract, septicaemia, and documented or suspected meningitis [3]. It is considered as one of the first choice antibiotics in the therapy of spontaneous bacterial peritonitis in cirrhosis [4]. Mechanism of action of cefotaxime was inhibiting bacterial cell wall synthesis. These antibiotics are active against a wide range of both gram-positive and gram-negative bacteria [5-6]. For the synthesis of cefotaxime, several methods have been employed. Rodriguez have been reported a rapid for the synthesis of cefotaxime, starting from the 7-ACA and a thioester of ATMA (MAEM), a commercially available reagent that does not need preliminary modifications to react [7]. Tippa and Singh reported a simple procedure for the synthesis of cefotaxime, using 7-ACA and diethyl thiophosphoryl [(*Z*)-(2-aminothiazol-4-yl)-2-(methoxyimino) acetate (DAMA) as starting material [8]. Those methods are not environmentally friendly because it used dichlorometane and chloroform as a solvent. As a general method for the synthesis of Cefotaxime, used most frequently has been direct coupling of 7-aminocephalosporanic acid (7-ACA) with S-benzothiazol-2-yl (2- amino- 4-thiazolyl) (methoxyimino) thioacetate (MAEM) developed by Lonza.

The purpose of this paper is to report an efficient procedure for the synthesis of cefotaxime, starting from the 7-ACA and MAEM, a commercially available reagent that does not need preliminary. The method has been used in this research is modified Macher method to increase the yield and purity of the product [9].

MATERIALS AND METHODS

Materials

7-ACA and MAEM (Hefey JOYE Import and Export Co., Ltd); TEA (Merck), Acetone (Merck), Methanol For Chromatography (Merck); HCl (Merck); Dichloromethane (Merck); Benzenesulphonic acid (Sigma); p-toluene-sulphonic acid monohydrate (Sigma); Nutrient agar (Merck); TFA (Merck); Cefotaxime Acid Refference Standard (European Pharmacopeia);

Synthesis of Cefotaxime Acid

The process was carried out at room temperature. Weighed 0.2 moles of 7-ACA in a mixture of water and acetone, mixed well to form a suspension. TEA was added at room temperature and stirred well. A solution is obtained within 20-40 minutes.). 2 moles of MAEM were added and further added acetone. The process monitored with intermittent samples. Starting material was checked using TLC after 1-2 hours and pH were adjusted 3.5 by using hidrocloric acid or Benzenesulphonic acid or p-toluene-sulphonic acid monohydrate. Acetone or dichlorometane was added dropwise and crystalline cefotaxime acid is separated by filtration. Crystalline cefotaxime was dried overnight in the vacuum drying chamber.

Antibacterial activity Confirmation

The antibacterial activities confirmation of cefotaxime acid was tested against a panel of bacterial pathogen, *Escherichia coli ATCC 25922, Salmonella typhimurium ATCC 14028, Bacillus subtilis* ATCC 633 and *Staphylococcus aureus* ATCC 25923. The tested compounds were dissolved in acetone to give a final concentration 10 ppm. Susceptible sterile discs were impregnated by the tested substance 10 µL via micropipette. The biological activity for each substance was tested on surface seeded nutrient agar medium

with the prepared susceptible discs. The positive control was chloramphenicol and the negative control using acetone. All bacterial strains used in this study were obtained from the bacterial culture collection of Biotechnology Center BPPT, Tangerang, Indonesia.

High Performance Liquid Chromatography

The HPLC analysis was accomplished using KNAUER®– ASI – 1998 – 2005. The used column was Inertsil ODS3, C8 (150 × 4.6 mm) packed with 5 µm particles. The injection volume, Twenty µL of the sample, was applied for all experiments in a gradient mobile phase containing methanol and 0.1% TFA (35 : 65) that pumped through the column with a flow rate of 1 ml/min. Furthermore, quantification was calculated at 254 NM using a PDA detector. Lastly, before employing in the machine, the mobile phase was filtered through a 0.45-µm membrane filter and degassed.

Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{1}HNMR spectrum of the Cefotaxime acid synthesized and Cefotaxime Acid CRS EP Reference was recorded using a JEOL 500MHz; NMR spectrometers chemical shifts are given in δ values (ppm) using TMS internal standard. Acetone was used as solvent.

Liquid Chromatography-Mass Spectrofotometry (LC-MS)

The LC-MS spectrum of of Cefotaxime acid synthesized was recorded using HPLC Alliance 2695 (Waters) with Photodiode Array Detector 2996 (Waters); Column Symmetry C18, 5 µm, 150x4.6 mm (Waters); Flow rate 1 ml/min; injection volume 10 µL; Room Temperature; Eluent: A. H2O + 0.1% formic acid, B. Acetonitrile + 0.1% formic acid; Gradient Method. MS: ESI-Tof-MS LCT Premier XE (Waters) Positive and negative mode: Capillary voltage 200 V; Sample cone voltage 60V; Desolvation T 300°C; Source T 120 °C; Desolvation gas 500L/h; Cone gas 10 L/h.

RESULTS AND DISCUSSION

The principle of the synthesis of cefotaxime is reacting 7-aminoacephalosporanic acid (7-ACA) with thioester reactant (Figure 1). 7-ACA is the core structure for the synthesis of cephalosporin antibiotics and can usually be obtained from the hydrolysis of cephalosporin C. chemoenzymatic. Thioester reactant is S-benzothiazol-2-yl(2-amino-4-thiazolyl) (methoxyimino) thioacetate (MAEM) or thioester of (*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (ATMA) [7-12].

A brief summary is shown in Table 1 of the different methods reported for the synthesis of cefotaxime acid, taking into account the reaction conditions, the reagents used and the yields obtained in each process, for comparison. Method C has the highest yield than other methods. Based on the analysis by HPLC, purity cefotaxime acid C method similar to the method A and D. However, the raw material method A were much cheaper than methods B and D, so that method A was more efficient than the other methods.

The synthesized material of Cefotaxime Acid was analyzed by High performance Liquid Chromatography (HPLC) and compared with the reference standard chromatogram for identification of the synthesized Cefotaxime Acid. HPLC chromatograms were given in Fig 2. The chromatography purity of the material was also verified by using the validated method of reference standard of Cefotaxime Acid and found the purity as 93.4% and 102%.

Identification of Cefotaxime Acid was also carried out by LC-MS and compared with reference standard chromatogram. Chromatogram LC closely with the reference standard shows a peak dominant with a retention time of 2.39 minutes. LC-MS mass spectrum ESI negative ion cefotaxime acid in Figure 3 shows the tallest peak at m / z (M-H +) 454.0491. The molecular formula of cefotaxime acid compound is C16H17N5O7S2 terdeprotonisasi expected. The calculated molecular mass is 455.0491 gram/mole. This is consistent with the calculated mass of cefotaxime acid reference standards with value 455 063 gram/mole

ISSN: 0975-8585

1H-NMR spectrum of the synthesized material of Cefotaxime Acid compared with the reference standard cefotaxime acid spectrum. Figure 4 showed the similarity of the 1H NMR spectrum of cefotaxime acid synthesized by the standard reference cefotaxime acid.

Escherichia coli, *Salmonella Thyposa*, *Bacillus subtilis*, and *Staphylococcus aureus* were used to confirm antibacterial activity as measured by the paper disc diffusion method. Test compound was dissolved in acetone to yield concentration.3125 ppm, 0.625 ppm, 1.25 ppm, 2,5 ppm, 5 ppm and 10 ppm. Cefotaxime acid reference standard CRS and Chloramphenicol were used as standards for the comparison of antibacterial activities.

The antibacterial of the synthesized material of cefotaxime acid against gram-positive and gram-negative organisms were similar to that of a reference standard. Table 2 and Table 3 showed both of the synthesized material of cefotaxime acid and the reference standard CRS of Cefotaxime acid have shown the antibacterial activity against *E coli, S thypimurium* and *S.aureas* and no inhibitory activity on the *S aureus*. Davis and Stout state that the inhibition zone of the agar diffusion test measuring less than 5 mm, it was considered a weak inhibitory activity, the inhibition zone measuring 5-10 mm is average and the inhibition zone was 10-19 mm and 20 mm are categorized strong or very strong categorized [11].

Table 1. Reported methods in synthesis of cefotaxime acid*)

METHOD	REACTANTS	SOLVENTS	Temperature, Time Reaction	YIELD (%)	Assay (%)
A	7-ACA, MAEM, TEA, HCL	Acetone	20-25ºC; 3-4 hours	67.46	102
B	7-ACA, MAEM, TEA, BSA	Acetone	10-15º, 18 hours	72.41	93.4
C	7-ACA, MAEM, TEA, p-TSAM	Acetone	15-20 ºC, 4-5 hours	82.4	102
D	7-ACA, MAEM, TEA, HCl	Dichlorometane	10-15 ºC, 1-2 hours	66.59	102
*)7-ACA, 7-aminocephalosporanic acid;MAEM, 2-mercaptobenzothiazolyl thioester of (*Z*)-2-[2-aminothiazol-4-yl]-2-methoxyimino acetic acid; BSA, benzenesulphonic acid; p-TSAM, p-toluene-sulphonic acid TEA, triethylamine monohydrate					

Table 2: Antibacterial screening of synthesized cefotaxime acid against *E. coli* and *S. typhimurium* (zone of inhibition in mm).

Sample	Incubation Time	*Escherichia coli* ATCC 25922						*Salmonela typhimurium* ATCC 14028					
		Sample Consentration (µg/mL)						Sample Consentration (µg/mL)					
		0.31	0.62	1.25	2.5	5	10	0.31	0.62	1.25	2.5	5	10
The Synthesized material of Cefotaxime Acid	24 hours	-	-	8.00	11.85	15.57	19.12	-	-	-	6.49	9.72	11.87
	48 hours	-	-	7.71	11.63	15.68	19.16	-	-	-	6.05	8.25	11.79
The Reference Standard CRS of Cefotaxime Acid	24 hours	-	-	7.95	11.81	16.39	18.7	-	-	-	6.65	9.86	12.61
	48 hours	-	-	7.45	11.41	15.69	19.0	-	-	-	6.03	8.14	12.29

ISSN: 0975-8585

Chlorampenicol (1000 µg/mL)	24 hours	20.9	-	-	-	-		23.82	-	-	-	-	
	48 hours	20.3	-	-	-	-		23.49	-	-	-	-	
Negative control (Acetone)	24 hours	-	-	-	-	-	-	-	-	-	-	-	-
	48 hours	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: Antibacterial screening of synthesized cefotaxime sodium derivatives and sulfonamides against *B. substilis* and *S. aureus* (zone of inhibition in mm).

Sample	Incubation Time	*Staphylococcus aureus ATCC 25923*						*Bacillus subtilis ATCC 6633.*					
		Sample Consentration (µg/mL)						Sample Consentration (µg/mL)					
		0.31	0.62	1.25	2.5	5	10	0.31	0.62	1.25	2.5	5	10
The Synthesized material of Cefotaxime Acid	24 hours	-	-	-	-	-	-	-	-	-	7.39	9.05	11.27
	48 hours	-	-	-	-	-	-	-	-	-	6.78	8.59	10.27
The Reference Standard CRS of Cefotaxime Acid	24 hours	-	-	-	-	-	-	-	-	-	7.41	9.27	10.26
	48 hours	-	-	-	-	-	-	-	-	-	6.91	8.75	11.15
Chloramphenicol (1000 µg/mL)	24 hours	-	-	-	-	-	-	18.28	-	-	-	-	-
	48 hours	19.11	14.86	-	-	-	-	16.54	-	-	-	-	-
Negative control (Acetone)	24 hours	-	-	-	-	-	-	-	-	-	-	-	-
	48 hours	-	-	-	-	-	-	-	-	-	-	-	-

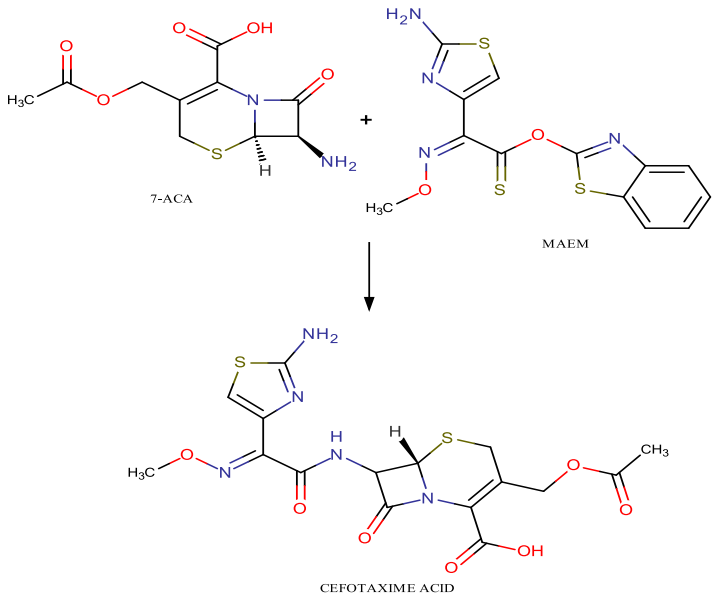
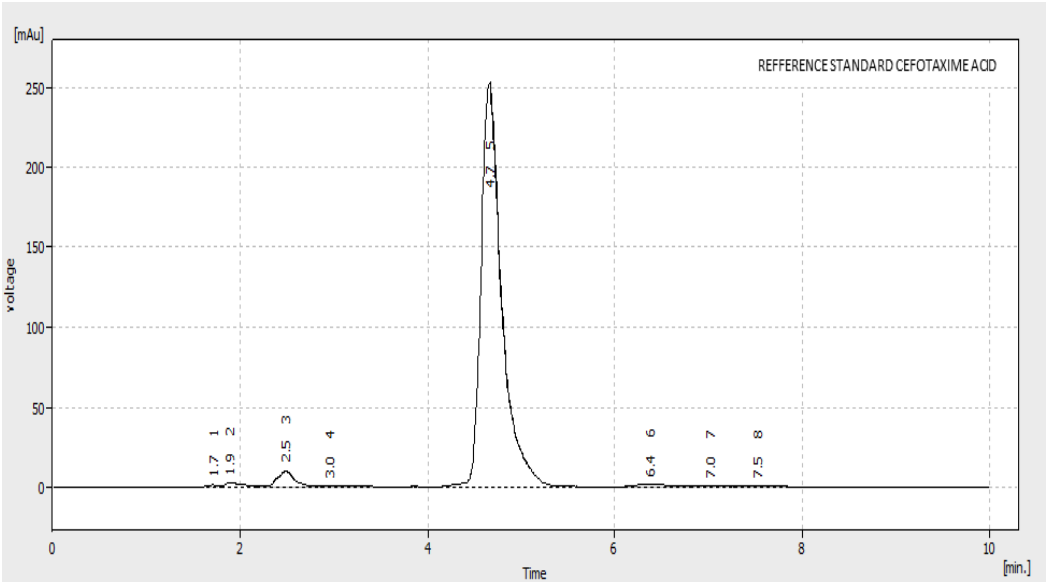
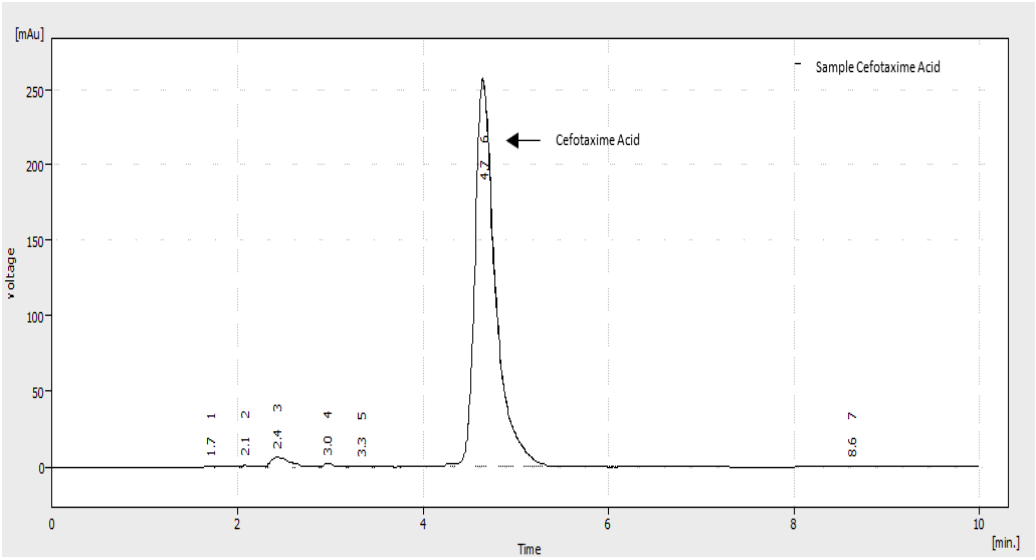


Fig. 1. Preparation of Cefotaxime Acid

ISSN: 0975-8585



(a)



(b)

Fig. 2 Chromatogram of (a) Cefotaxime acid reference standard and (b) the synthesized Cefotaxime acid

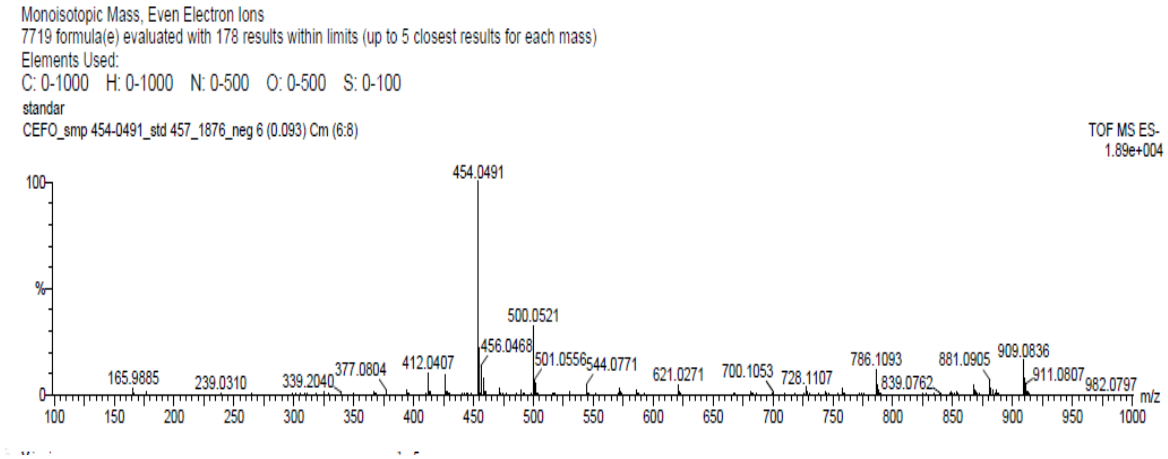
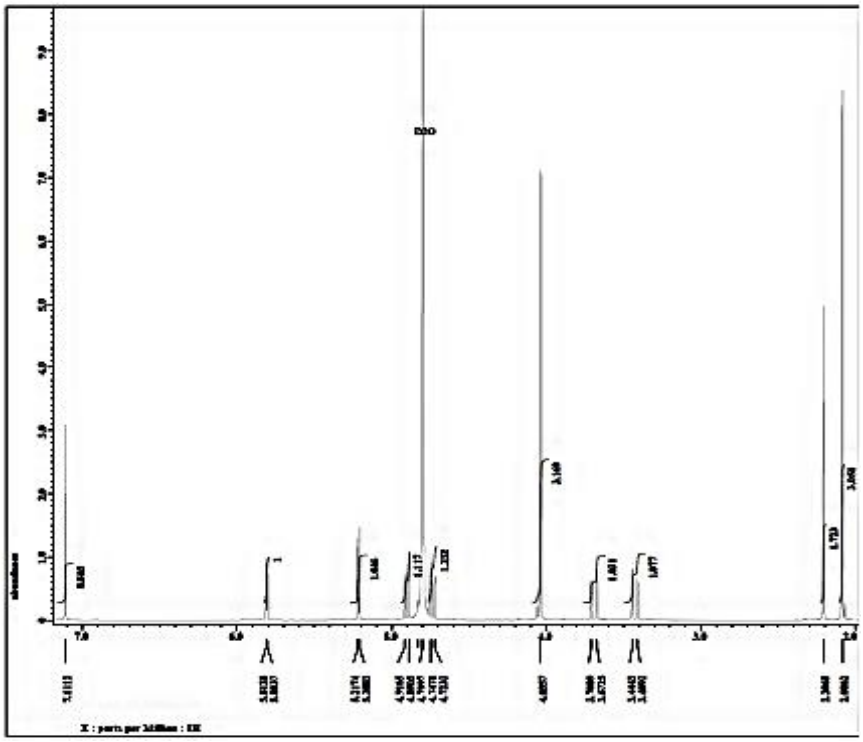
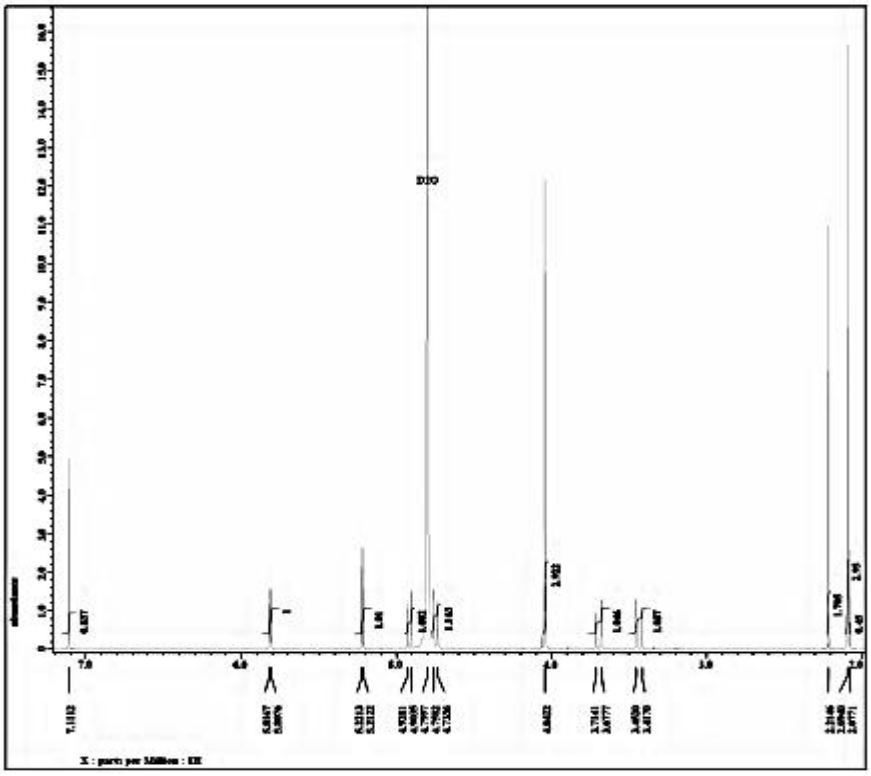


Fig. 3 LC-MS Spectrum of Cefotaxime acid

ISSN: 0975-8585



(a)



(b)

Fig 4. ^{1}HNMR spectrum of (a) Reference Standard of Cefotaxime Acid and (b) the synthesized material of Cefotaxime Acid

ISSN: 0975-8585

CONCLUSIONS

This work shows that the synthesized material of cefotaxime acid has similar caracter with the refference standard of cefotaxime acid. But still required optimization method to obtain high yield results

REFERENCES

[1] Anonim, 2014, Direktorat Bina Produksi dan Distribusi Kefarmasian, Ditjen Bina Kefarmasian dan Alat Kesehatan, Kementerian Kesehatan Republik Indonesia.

[2] Instruksi Presiden Republik Inonesia Nomor 6 Tahun 2016 Tentang Percepatan Pengembangan Industri Farmasi dan Alat Kesehatan.

[3] Stirbet, D, litescu, S-C., and radu G-L., J. Serb. Chem. Soc., 2014; 79 (5) ; 579–586 JSCS–4609

[4] Sheela Joshi S., Shukla A., Jhala R.S., Arabian Journal of Chemistry, 2015; http://dx.doi.org/10.1016/j.arabjc.2014.12.031

[5] Nuevas L., Gonza´lez, R., Rodrı´guez, J.C., Hoogmartens, J., , *J. Pharm. Biomed. Anal.*, 1998; 18 ; 579–583

[6] Morelli, B., Journal of Pharmaceutical and Biomedical Analysis, 2003; 32; 257- 267

[7] Rodriguez JC, Hernandez R., Gonales M., Lopez A.L.,Dini, A., II. Farmaco., 2000; 55; 393-396.

[8] Macher, W., Patent, World Intellectual, Property Organization, 1996; WO 96/20198

[9] Durga Mallikarjuna Rao Tippa* and N. Singh International Journal of Pharmaceutical Science and Research, 2011; Vol. 2; Issue 8; 2178-2182

[10] Yoon, M.-Y., Lee, H. B. and Shin, H., Bulletin of the Korean Chemical Society 2011; 32; 407-410.

[11] Durga Mallikarjuna Rao Tippa and N.Singh. 2011. Synthesis of Cefotaxime From DAMA (Diethyl Thiophosryl [(S)-(2-Aminothiazol-4-YL)-2-(Methoxyyimino)and 7-ACA (7-Amino Cephalosporinic Acid). International Journal of Pharmaceutical Sciences and Research, 2(8), 2178-2182

[12] Man-Young Yoon, Hee Bong Lee and Hyunik Shin. 2011. Development of an Isolable Active Ester, Diethyl Thiophosphoryl [(Z)-(2-Aminothiazol-4-yl)-2-(Methoxyimino)acetate (DAMA) for the Synthesis of Cefotaxime. Bulletin of the Korean Chemical Society, 32, 407-410.