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Efficient Procedure For Synthesis Of Cefotaxime Sodium Using Sodium-2-Ethylhexanoate As The Source Of Sodium Ion.

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ABSTRACT

Cefotaxime sodium is an antibiotic that has high economic value as well as the necessary but expensive. This research aims to find an efficient procedure for the synthesis of cefotaxime sodium. In this research, cefotaxime sodium was synthesized from cefotaxime acid and sodium-2-ethyl hexanoate. The optimal conditions for the synthesis were obtained as following: mole cefotaxime acid/mole sodium-2-ethyl hexanoate = 2:3; ethanol food grade as a solvent, reaction time at 10 minutes and room temperature. The yield of cefotaxime sodium was 97.11% based on cefotaxime acid and the purity was 99.10%. The chemical structure and molecular weight of products were identified with FTIR, NMR, and LC – Mass Spectrometry. The quality product testing is also done with the parameters solubility product in aqueous and acid media, absorbance value, pH in aqueous media, and purity. The products meet the requirements of the United States Pharmacopeia, Japan Pharmacopeia, and British Pharmacopeia.

Keywords: Cefotaxime sodium, efficient procedure, sodium-2-ethyl hexanoate, Pharmacopeia

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INTRODUCTION

Cefotaxime is a third-generation parenteral cephalosporin antibacterial in the treatment of infections due to susceptible Gram-positive and Gram-negative bacteria. The antibiotic displays a high antimicrobial potency, a broad antibacterial spectrum, high resistance against the action of beta-lactamases, as well as a low index of side effects and lowered toxicity profile compared to predecessors [1]. Cefotaxime sodium is necessary to meet the needs of antibiotics in the country is very large. The Indonesia sales values of this antibiotic in 2015 was IDR 308,252,138,350, -.

Cefotaxime sodium has wide clinical applications for the treatment of infections of the respiratory tract, gynecologic, skin, bone and joint, urinary tract, septicemia, and documented or suspected meningitis [2]. It is considered as one of the first choice antibiotics in the therapy of spontaneous bacterial peritonitis in cirrhosis [3]. The 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 [4-5].

Cefotaxime is a semi-synthetic cephalosporin of an acetyl side-chain on aminothiazolyl ring and an alpha-syn-methoximino group [6]. The molecular formula is C16H16N5NaO7S2 and molecular weight is 477 gram/mol. Its IUPAC name is sodium (6R,7R)-7-[(Z)-2-2(2-amino-4-thiazolyl)-2-(methoxyimino) acetamido]-3-acetyl-oxymethyl-3-cephem-4-carboxylate. The synthesis of Cefotaxime used most frequently was a direct coupling of 7-aminocephalosporanic acid (7-ACA) with S-benzothiazole-2-yl(2-amino-thiazolyl)(methoxyimono)thioacetate (MAEM) developed by Lonza [7].

Several methods for the synthesis of cefotaxime sodium have been developed using not environmentally friendly solvent and time-consuming. Tippa and Singh (2011) have been reported a simple procedure for the synthesis of cefotaxime sodium, starting material from cefotaxime acid and sodium 2-ethyl hexanoate using methanol or ether as a solvent for 10 hours reaction [7]. Zang (2010) reported an alternate procedure for the synthesis of cefotaxime sodium, using cefotaxime acid and sodium acetate as starting material. These methods used 2-propanol and chloroform as a solvent and were needed 5 hours for synthesizing [8]. Patent WO96/20198 was reported as synthesizing of cefotaxime sodium that was used sodium acetate trihydrate was a source of sodium ion using acetone as a solvent. That process needs 5 hours for getting sodium cefotaxime [9]. US patent Pub No.: US 2007/0004916 A1 was converted cefotaxime acid into sodium salt for more than 2 hours using sodium-2-ethyl hexanoate in of ethyl acetate, methanol and triethylamine to obtain cefotaxime sodium [10]. In this report, we present the development method for synthesizing cefotaxime sodium using a more efficient and environmentally friendly solvent.

MATERIAL AND METHODS

Materials

Cefotaxime acid and ethy hexanate were purchased from Hefei China. Ethanol food grade from Indonesian. Sodium cefotaxime USP standard was purchased from sigma Aldrich, Methaol HPLC grade and acetonitrile HPLC grade were purchased from merck.

Synthesis of Cefotaxime Sodium

A source of sodium ion was sodium-2-ethyl hexanoate. Sodium-ion added in an equivalent amount or an excess, for example in a ratio 1:1, 1:2, and 2:3. Seed crystal of cefotaxime sodium was added after the reaction of sodium 2-ethyl hexanoate and cefotaxime acid. Ethanol was added to complete crystallization. This process used several ethanol qualities such as food-grade ethanol, pro analysis grade ethanol and recycled ethanol. The reaction temperature was set at 10° C and room temperature and time of reaction were 10, 30 and 60 minutes. Crystalline cefotaxime sodium was dried overnight in the vacuum drying chamber

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 sodium was synthesized and cefotaxime sodium 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 the solvent.

Liquid Chromatography-Mass Spectrophotometry (LC-MS)

The LC-MS spectrum of cefotaxime sodium 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. H_2O and 0.1% formic acid, B. Acetonitrile and 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.

RESULT AND DISCUSSION

A route of synthesis of cefotaxime sodium using cefotaxime acid and sodium-2-ethylhexanoate as raw material is shown in Figure 1.Once of the benefit using of sodium-2-hexanoate as a source of sodium ion that it was dissolved in ethanol. The solvent was also well known as environmentally friendly, non-toxic and low cost.

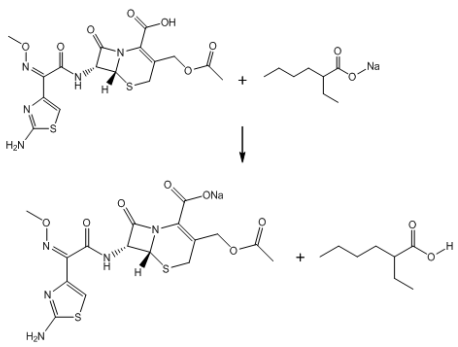

Fig. 1. Synthetic Route of Cefotaxime Sodium

Cefotaxime is poorly soluble in water, therefore for clinical used, it is transformed into soluble sodium salt. Sodium cefotaxime prepared by reaction with salts carboxylic acids of higher pK. Different water alcoholic mixtures are used as reaction media to isolate ad purify products [11]. In this research, the effect of the mole ratio reactants, types of ethanol solvent, amount of ethanol, time of reaction, the addition of seed and temperature of reaction on the synthesis of cefotaxime sodium have investigated. The quality of the product must be appropriated with pharmacopeia standards such as USP, BP, EP, and JP.

Table 1. Standard parameter of cefotaxime sodium at several of pharmacopeia

Standard Parameters	USP	BP	EP	JP
Solubility in Water	Clear	Clear	Clear	Clear
Solubility in Acid	Clear	Clear	Clear	-
pH	4,5-6,5	4,5-6,5	4,5-6,5	4,5-6,5
Absorbance	<0,2	<0,4	<0,4	-
Assay (HPLC)	96-102%	96-102%	96-102%	91.6%

The compositions of the mole ratio of cefotaxime acid with sodium-2-ethyl hexanoate were studied in a ratio 1:1, 1:2 and 2:3. The results of the effect of the mole ratio of the reactants on cefotaxime sodium product

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are shown in Table 2. The optimal condition for the synthesis of cefotaxime sodium was obtained at mole cefotaxime acid/mole sodium-2-ethylhexanoate=2:3 with yield = 87.18 % and assay = 102.88 % respectively.

Table 2. Effect of the mole ratio of reactants on the synthesis of cefotaxime sodium

Mole ratio of Cefotaxime Acid/ Na-2- ethylhexanoate	Yield (%)	Solubility in water	Solubility in Acid	pH (25°C)	Absorbance	Assay %
1:1	37.61	Clear	Clear	5.37	0.064	96.41
1:2	84.11	Clear	Clear	5.69	0.076	99.94
2:3	87.18	Clear	Clear	5.43	0.211	102.88

In the synthesis of cefotaxime sodium, three types of ethanol solvent have been used. The results of the effect of type ethanol solvent on cefotaxime sodium products are shown in Table 3.

Table 3. Synthesis of cefotaxime sodium using different ethanol grade

Solvent	Yield (%)	Solubility in water	Solubility in Acid	pH (25°C)	Absorbance	Assay (%)
Ethanol p.a	79.94	Clear	Clear	5.62	0.064	93.14
Ethanol food grade	75.68	Clear	Clear	5.41	0.136	95.92
Recycled ethanol	54.70	Clear	Clear	5.33	0.101	95.20

The optimal condition for the synthesis of cefotaxime sodium was occurred by using food grade ethanol with yield = 75.68 % and assay = 95.94 % respectively. Even the yield produced by ethanol p.a is greater than ethanol food grade produced, but the assay of ethanol food-grade is better. otherwise, the price of ethanol p.a is more expensive than ethanol food-grade. so, it is recommended to use ethanol food-grade as the chosen solvent to synthesize the cefotaxime sodium.

The effect of the amount of ethanol food-grade at the crystallization process of cefotaxime sodium was also investigated. The results of the effect of the amount of the solvent on cefotaxime sodium products are shown in Table 4. The optimal condition for the synthesis of cefotaxime sodium has occurred at 150 mL of ethanol food grade with yield = 72.53 % and assay = 97.6 % respectively.

Table 4. Effect of amount of ethanol on the synthesis of cefotaxime sodium

Amount of Ethanol (mL)	Yield (%)	Solubility in water	Solubility in Acid	pH (25°C)	Absorbance	Assay %
200	54.70	Clear	Clear	5.33	0.101	95.21
150	72.53	Clear	Clear	5.69	0.076	97.6x

Table 5 shows the result of cefotaxime sodium product with several of time reaction and amount of seed addition. The results are shown in Table 5 which the optimal condition for the synthesis of cefotaxime sodium occurred at the time of reaction 10 minutes and seed addition 1 gram, respectively. Ratio mole cefotaxime acid and sodium-2-ethyl hexanoate were 2:3. The yield and purity of cefotaxime sodium was 97.11 % and 99.11 %, respectively.

Table 5. Effect of Time reaction and seed addition on cefotaxime sodium product

Time Reaction (minutes)	Yield (%)	Solubility in water	Solubility in Acid	pH (25°C)	Absorbance	Assay %
10	86.69	Clear	Clear	5.43	0.103	100.62
30	87.18	Clear	Clear	5.25	0.104	100.88
60	86.50	Clear	Clear	5.13	0.110	98.64
10 (+1%Seed)	97.11	Clear	Clear	5.87	0.172	99.11
30 (+1% Seed)	84.11	Clear	Clear	5.64	0.190	99.94

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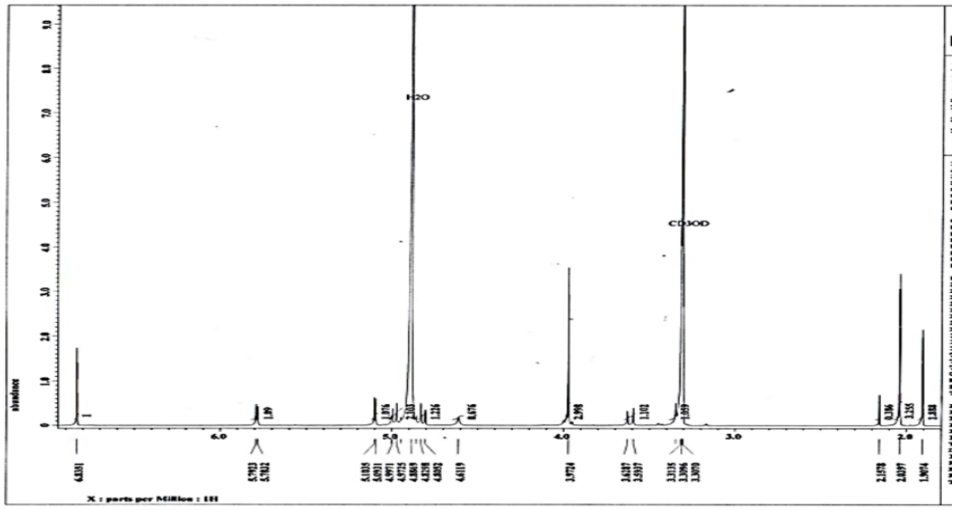
60 (+1% Seed)	87.xx	Clear	Clear	5.13	0.110	98.64

Table 6 shows the result of cefotaxime sodium product with two different temperature reaction with ratio mole of cefotaxime acid and sodium-2-ethyl hexanoate was 1:1. The optimal temperature for the synthesis of cefotaxime sodium was occurred at room temperature with yield and purity more higher compared to 10°C.

Table 6. Effect of temperature reaction on the synthesis of cefotaxime sodium

Temperature Reaction (°C)	Yield (%)	Solubility in water	Solubility in Acid	pH (25°C)	Absorbance	Assay %
10	54.70	Clear	Clear	5.33	0.101x	95.21
Room temperature	86.69	Clear	Clear	5.43	0.103x	102.62

The chemical structure of cefotaxime sodium was characterized by 1 H NMR and FTIR. Figure 2 a - b shows the spectrum of 1H NMR of cefotaxime sodium as-synthesized and cefotaxime sodium standard, respectively.



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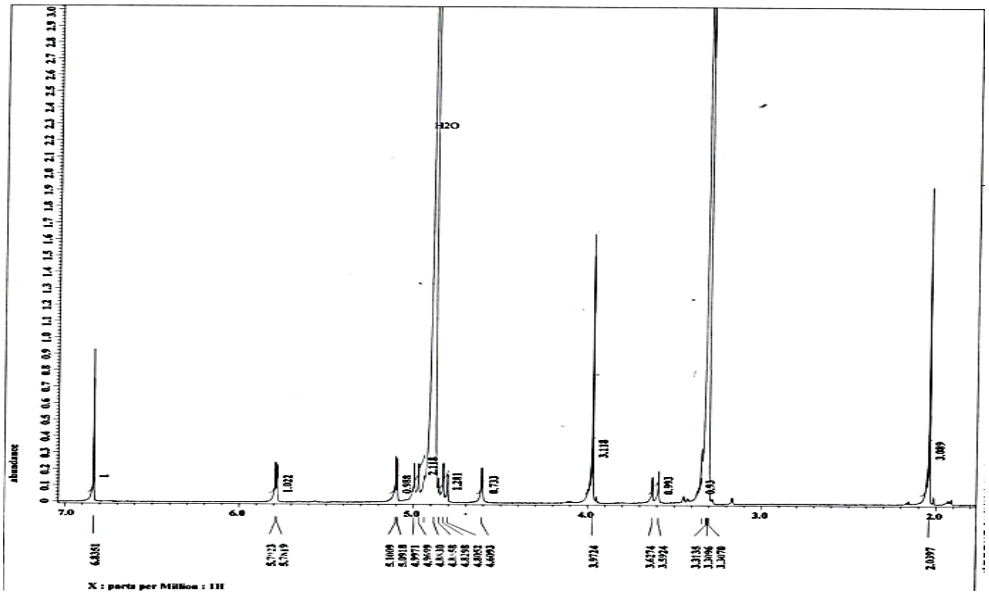

b

Fig 2. Comparative Spectrum 1H NMR of Cefotaxime Sodium Product (a) and Cefotaxime Sodium Standard (b)

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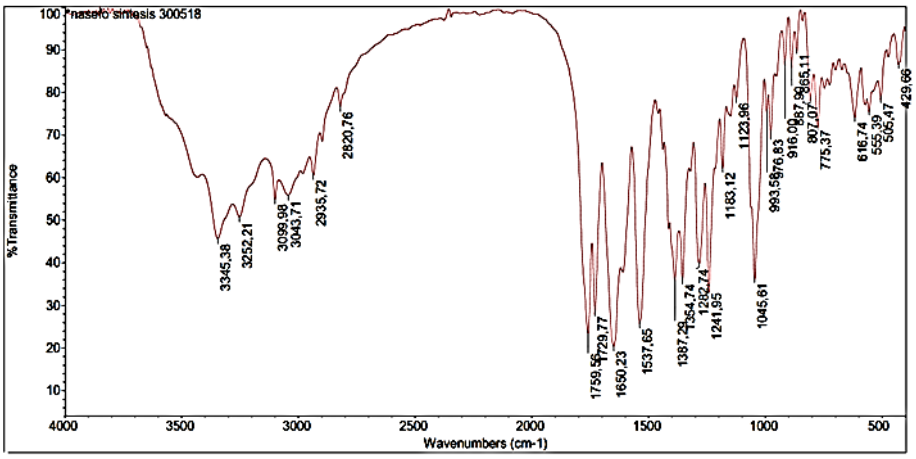
The cefotaxime sodium as-synthesized and cefotaxime sodium china products were characterized by FTIR spectroscopy. Figures 3.a and 3.b show that both of the FTIR spectra were identical concerning the main peaks of the cefotaxime component. Both compounds exhibit peaks assigned to the following groups: ketone, amine, ketone carboxyl.



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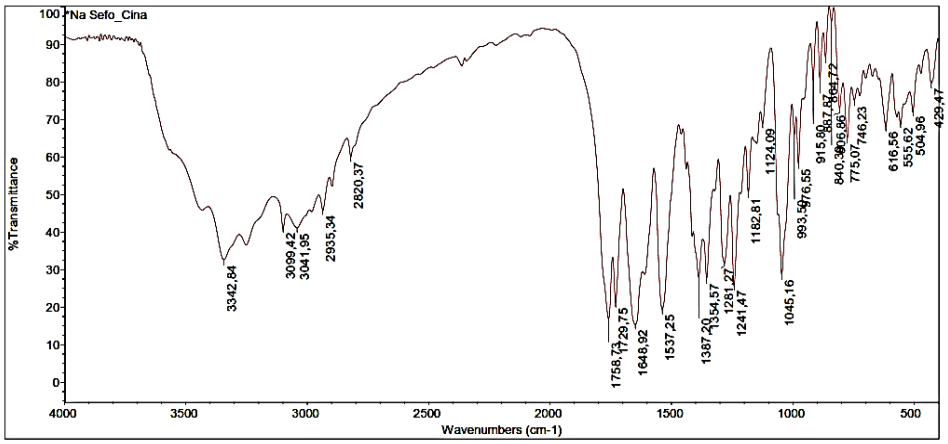

b

Fig 3. Spectra of FTIR of cefotaxime sodium as-synthesized and cefotaxime sodium China Product

Table 7. Comparative FTIR Spectral Data for Cefotaxime Sodium Product

IR (KBr) absorption bands (cm-1)			
Cefotaxime Sodium Product Synthesis		Cefotaxime Sodium Product China	
3345.38	NH stretch	3342.84	NH stretch
2935.72	Aliphatic C-H stretch	2935.34	Aliphatic C-H stretch
1759.56	C=O (lactam) stretch	1758.71	C=O (lactam) stretch
1729.77	C=O (carboxylic ester)	1729.75	C=O (carboxylic ester)
1650.23; 1537.65	C=C & C=N stretch	1648.92; 1537.25	C=C & C=N stretch
1387; 1354	Aliphatic C-H bend	1387; 1354	Aliphatic C-H bend
1045	C-O stretching	1045	C-O stretching

Table 7. Comparative FTIR Spectral Data for Cefotaxime Sodium Product

Table 7 show spectral data FTIR of Cefotaxime sodium as synthesized andcefotaxime sodium china product. The spectra data are similar to FTIR spectra data from Kumar et al (2010), which showed the existence

of functional groups as follows:NH[3430, 3347 (s,br)]; Aliphatic C-H[2938 (m)]; C=O from lactam[1760 (s)]; C=O from carboxylic ester [1729 (s)]; C=C & C=N stretch [1647, 1536 (s)]; Aliphatic C-H bend [1386, 1355 (s)] and C-O stretching [1062 (s)] [12].

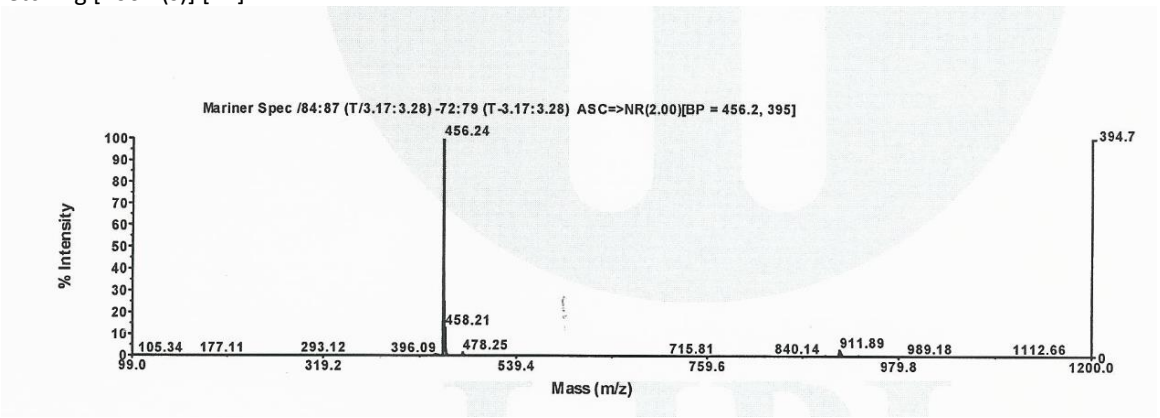

Fig 4. Spectrum of LC-MS/MS of cefotaxime sodium

The product solutions of cefotaxime sodium were analyzed by LC-MS system using a direct injection probe with ESI and APCI interfaces. From the mass spectrum recorded, the detection molecular ion selected was for cefotaxime sodium as 456.24 m/z. Typical mass spectrum and chromatogram of cefotaxime sodium is given in Fig 4.

CONCLUSION

Cefotaxime sodium was prepared from starting material cefotaxime acid and sodium 2-ethyl hexanoate using ethanol as the solvent. The efficient procedure for the synthesis of cefotaxime sodium could be reached in the suitable condition such as the mole ratio of reactants, temperature, reaction time, seed addition and the amount of ethanol food-grade at crystallization process. This method can be used to answer the environment problem for reducing unfriendly solvent and time-consuming.

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