BOOK OF PROGRAM

ISNM 2017

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Sustainable Use of Natural Products for Human's Health and Welfarea

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Bogor, Indonesia

Hosted by:

- **Tropical Biopharmaca Research Center IPB**
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- **The Indonesian Association of Natural Drugs Researchers**

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Abstract Submission Deadline:

~~July 14, 2017~~ July 31, 2017

Acceptance Notification:

~~July 24, 2017~~ August 5, 2017

Full-Paper Submission Deadline:

August 18, 2017

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ORAL PRESENTATION SCHEDULE

Day 1, Thursday, 24 August 2017
Time : 14.30 – 17.30
Room : Paralel 2 (Meeting Room B)

Time	Abstract / Code	Presenter & Title	
14.30-14.45	Abstract_019/ OP 10	Nabilah Amany	Induced Mutation By Gamma Ray Irradiation to *Rauvolfia serpentina* (L). Benth. ex. Kurz
14.45-15.00	Abstract_034/ OP 11	Gunawan Pasaribu	Ethnomedicine, Phitochemical, And Toxiticy Activity Of Several Medicinal Plants From Sebangau National Park, Center Borneo
15.00-15.15	Abstract_070/ OP 12	Nikmatul Ikhrom Eka Jayani., S.Farm., M.FarmKlin., Apt.	Effect of Drying Methods and Age of Leaves on Total Tannin of *Tectona grandis* L. and *Muntingia calabura* L.
15.15-15.30	Abstract_115/ OP 13	Prof. Dr. Dyah Iswantini, M.Sc.Agr	Antioxidant Biosensor on Superoxide Dismutase from Indonesia Microbes Immobilized in Indonesia Natural Zeolite
15.30-16.00	**Break**		
16.00-16.15	Abstract_077/ OP 14	Siti Karimah	Thin Layer Chromatography Fingerprint Analysis of Jati belanda (*Guazuma ulmifolia*) Leaves
16.15-16.30	Abstract_089/ OP 15	Dr Wulan Tri Wahyuni	Molecularly Imprinted Polymer Modified Carbon Paste Electrode for Voltammetric Detection of Quercetin
16.30-16.45	Abstract_099/ OP 16	Maximus M. Taek, M.Si	Ethnomedicinal Plants Used for the Treatment of Malaria in Malaka, West Timor
16.45-17.00	Abstract_120/ OP 17	Hendra Wijaya, Dr	Rapid Detection of Squid Allergy Using Paddle-Style Dipstick
17.00-17.15	Abstract_133/ OP 18	Dr. Enih Rosamah, M.Sc	Knowing Borneo Medicinal Plants For Skin Diseases
17.15-17.30	Abstract_093/ OP 19	Tatik Raisawati	Morphological of leaves and total flavonoid content of some accessions of *Sonchus arvensis* L.

ORAL PRESENTATION SCHEDULE

Day 1, Thursday, 24 August 2017
Time : 14.30 - 15.30
Room : Paralel 1 (Ballroom 3)

Time	Abstract / Code	Presenter & Title	
14.30-14.45	Abstract_002/ OP 01	Muhammad Thorieq	Belutidine: Eel mucus ointment as wound healing therapy
14.45-15.00	Abstract_004/ OP 02	Karina Citra Rani, S.Farm., M.Farm., Apt	Formulation and Characterization of Atenolol-β-Cyclodextrin Orally Disintegrating Tablets
15.00-15.15	Abstract_046/ OP 03	Muhammad Farid Rizal	Modified Electro-Acupuncture Technology And Standardized Extracts Of Dragon Fruits As An Immunomodulator Of Canine Parvovirus
15.15-15.30	Abstract_090/ OP 04	Lilis Nurhadijah	Activities of Jatropha Seed and Pare Formulation on the Spermatogenesis of Wistar Rats

Formulation and Characterization of Atenolol-β-Cyclodextrin Orally Disintegrating Tablets

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ABSTRACT

Atenolol is an antihypertensive drug and has been widely used in hypertension therapy. The mechanism of atenolol is antagonist of competitive beta (1)-selective adregenergic receptor. Atenolol has a low solubility characteristic in water and gastric fluid. The rate of absorption is often controlled by the rate of dissolution for poorly soluble drugs. In this study, the solubility of atenolol has been increased by inclusion complex using β-cyclodextrin made by several methods (physical mixture, kneading, and solvent evaporation). Evaluation and characterization of atenolol-β-cyclodextrin inclusion complex consists of drug content, dissolution profile, Fourier Transformed Infrared analysis (FT-IR), Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), and Scanning Electron Microscope (SEM). The results of the drug content analysis, dissolution test, and characterization showed that atenolol-β-cyclodextrin inclusion complex, which has been made by solvent evaporation method was the best composition. Therefore, a solvent evaporation method was chosen to prepare orally disintegrating tablets of atenolol-β-cyclodextrin using direct compression technique. Orally disintegrating tablets of atenolol-β-cyclodextrin were prepared using crospovidone as disintegrant. The results of pre-compression test and post-compression test revealed that orally disintegrating tablets of atenolol-β-cyclodextrin inclusion complex had good physicochemical characteristics and met quality requirements.

Keywords: atenolol, inclusion complex, β-cyclodextrin, orally disintegrating tablets.

INTRODUCTION

Atenolol is a competitive β(1) -selective adrenergic antagonists and has been widely used in hypertension therapy. Atenolol is one of the most frequently used β-blockers in the treatment of cardiovascular disease, because of its anti-hypertensic and anti-arrhythmic properties (Sweetman, 2009). Atenolol is slightly soluble in water. The solubility of atenolol in water (25°C) is approximately 13.3 mg/ml (Sweetman, 2009; Florey, 1984). Low solubility characteristic of atenolol in water and gastric fluid caused the bioavailability of atenolol is about 50% following oral administration (Sweetman, 2009). The rate of absorption of the drugs which have low solubility characteristic is often controlled by the rate of dissolution (Shargel and Pong, 2004). The rate of dissolution of low solubility drugs can be increased by several methods such as decrease the particle size using micronization and nanoparticle techniques, solid dispersion, salt formation, co-crystal, inclusion complex, etc. (Sinko, 2006; Kumare et al., 2013). Host-guest complexes are used in the pharmaceutical industry to improve the bioavailability, masking unpleasant taste, and improve the stability of drugs in aqueous solutions (Pop et al., 2002; Borodi et al., 2008). As host molecules, commonly cyclodextrins are used. Cyclodextrins (CDs) are toroidal-shaped cyclic oligomers of α-(1,4)-D-glucopyranose units which contribute to several guests-associated phenomena in solution (Buha et al., 2012). Cyclodextrins are cyclic oligosaccharides formed in 6 (αCD), 7 (βCD), or 8 (ϒCD) (α-1,4) -linked D-glucopyranose units. Cylodextrins with more than eight glucopyranose units do exist but are of limited interest as drug solublizers and stabilizers (Douroumis et al., 2013).

Cylodextrins are organic compounds which have cyclic toroidal shaped. The arrangement of monomers in the cyclodextrin molecules can be described as a ring, a doughnut, a cylinder, or more precisely a truncated cone. The structure consist of a hydrophilic exterior and the

hydrophobic exterior. The hydrophobic exterior structure caused by the hydrophobic carbon backbones of glucopyranose monomers which construct the structure of β-cyclodextrin (Douroumis et al 2013; Kurkoy et al., 2012). Because of the cyclic structure of cyclodextrins molecules, it was proposed that cyclodextrins should entrap a molecules in their cavity (Kurkoy et al., 2012). Through complex formation, the solubility of the drugs can be enhanced.

The results from the previous study stated that β-cyclodextrins has been widely used in the pharmaceutical industry because of the ease of production and subsequent low price (Kurkoy et al., 2012). The ability of β-cyclodextrins to produce inclusion complex compound is highly affected by the size, shape, and the hydrophobic nature of the guest molecules (Prabhu et al., 2012). Inclusion complex of drugs with cyclodextrin has been extensively studied due to its pharmaceutical interaction. The inclusion complex of drug molecules with β-cyclodextrins cuased modification of pharmacokinetic properties of drug molecules such as (i) increase the dissolution of low solubility drugs, improves production efficiency, chemical stability, and bioavailability of poorly water soluble drugs, (iii) caused the reduction of drug toxicity, (iv) control the release profil of the drug. β-cyclodextrin has been widely used in pharmaceutical technology because of big molecular cavities. Internal cavity of β-cyclodextrin is 6 Å. The big molecular cavity of β-cyclodextrin increase the possibilities of the drug which will be entrapped in the cavity. In general, one molecule of cyclodextrin will trap one molecule of drug in cylcodextrin's cavity (Nikolic et al, 2007).

Inclusion complexation of cyclodextrin with guest molecules are produced because of hydrophobic interactions, electronic effects, van der Walls forces, and steric factors. Moreover, the formation of hydrogen bond in the rim cavity also play an important role (Iacovino et al., 2012). An inclusion complex of atenolol with β-cyclodextrin enhance the aqueous solubility of atenolol and dissolution rate of atenolol. Moreover, an inclusion complex of atenolol with β-cyclodextrin also enhance pre gastric absorption of atenolol (Shankarrao et al., 2010). Inclusion

complex of atenolol with β-cyclodextrin in 1:1 ratio has been chosen because of the evidence from previous studies revealed improvement in the dissolustion aspect of atenolol (Borodi et al, 2007). In this study, inclusion complex of atenolol with β-cyclodextrin were prepared using 1:1 ratio.

The novelty of this research was to improve the solubility and dissolution characteristic of atenolol using material engineering. Material engineering technique which has been done in this study was inclusion complex The objective of this study is to prepare inclusion complexes of atenolol with β-cyclodextrin by different methods such as physical mixture, kneading, and solvent evaporation method to increase the dissolution of atenolol. Inclusion complex of atenolol with β-cyclodextrin are characterized by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRD), fourier transform infrared (FT-IR), and scanning electron microscopy (SEM). The inclusion complex then formulated into orally disintegrating tablets to increase patient compliance, especially in geriatric patients.

Orally disintegrating tablets are tablets which have high porosity, low density, and low hardness Orally disintegrating tablet will produce smooth suspension in the oral cavity when this tablet disintegrate. Absorption can occur in the oral cavity, because there are a lot of blood vessel in there. Tablets were prepared by using crospovidone as disintegrant, aspartam, mannitol direct compress, Avicel PH 102®, mint flavour, magnesium stearate, and Aerosil®. Crospovidone was added to facilitate drug release and consequently improve the solubility of the drugs (Amrutkar et al, 2007). Tablets were prepared by using direct compression technique. Direct compression technique is a simple and cost effective compare to the granulation technologies (Shankarrao et al., 2010).

MATERIAL AND METHODS

Materials

Materials that were used in this study consists of atenolol pharmaceutical grade (p.g) (Refarmed Chemicals, Lugono Switzerland), β-cyclodextrin (Roquette, France), etanol (EtOH) pro analysis (p.a) (Merck), *crospovidone* (Kollidon® CL) p.g (BASF South East Asia Pre-Ltd), magnesium stearate p.g (Faci Asia Pacific PTE LTD), aspartame f.g (Ajinomoto Co. Inc.), aqua demineralisata (Laboratorium of qualitative chemistry University of Surabaya), mannitol direct compress grade p.g (Roquette Freses, France), colloidal silicon dioxide p.g (Brataco), mint flavor f.g (KH Roberts), sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) p.a (Merck), disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$) p.a (Merck), natrium asetat trihidrat (CH_3COONa) p.a. (Riedel), acetic acid glacial (CH_3COOH) p.a (Merck), metanol (MeOH) pro HPLC (Mallinckrodt Chemicals), Microcrystalline cellulose (Avicel PH 102®) p.g (Mingtai Chemical Co. LTD), talk (Brataco), and filter paper No 41 (Whatmann®)

Preparation of atenolol-β-cyclodextrin inclusion complex

Preparation of physical mixture

Inclusion complex of atenolol-β-cyclodextrin was made by kneading and co-precipitation method. The physical mixture of atenolol and β-cyclodextrin in 1:1 molar ratio (molar/molar) was prepared by mixing atenolol and β-cyclodextrin. Atenolol and β-cyclodextrin were previously sieved through sieve no. 60 before the mixing process (Gite et al., 2014).

Kneading method

Atenolol and β-cyclodextrin in 1:1 (molar/molar) were mixed until produce homogenous mixture. Etanol was added to the mixture of atenolol and β-cyclodextrin. Atenolol and β-cyclodextrin were kneaded like a slurry by using mortar and a stamper. After 4 hours of grinding, the solvent had been evaporated from the slurry consistency. This paste was dried in a tray dryer at 50°C and the formation of powder like complex had been obtained. The dried complexes were powdered, then passed through sieve no.60. The inclusion complex of

atenolol and β-cyclodextrin then stored in air tight container (Gite et al., 2014; Ghosh et al., 2013).

Solvent evaporation method

In this technique, atenolol was dissolved in ethanol and β-cyclodextrin was dissolved in the water. The ratio of atenolol and β-cyclodextrin was 1:1 (molar/molar). This mixture was mixed thoroughly by using magnetic stirrer for 2 hours, then this mixture was placed in a water bath (90 °C) to evaporate the solvent. The inclusion complexes were obtained as a crystalline powder pulverized. The inclusion complexes were sieved by using siever no. 60 and stored in air tight containers till further use (Gite et al., 2014).

Characterization of atenolol-β-cyclodextrin inclusion complex

Fourier transform infrared spectrophotometry

Infrared spectrum of atenolol, physical mixture, and inclusion complex which was obtained by using kneading method and solvent evaporation method were recorded using the KBr method. The infrared spectrum of the samples were prepared by KBr disc over the wave number of 4000 to 400 cm^{-1} by using FTIR (Florey 1984; Gite et al., 2014).

Differential scanning calorimetry

Differential scanning calorimetry were used to confirm the formation of inclusion complex. The sample, consist of atenolol, physical mixture of complex, and inclusion complex was weighed and heated at a scanning rate of 10°C/minute between 40 and 200°C. The flow of nitrogen was 40 ml/min of nitrogen flow (Gite et al., 2014).

Analysis of crystallographic aspect using powder X-ray diffraction (XRD)

X-ray diffractometer were used to obtain the powder x-ray diffraction of atenolol, physical mixture, and inclusion complex. X-ray diffractometer (Phillips) was arranged in the range 2Θ (5-50°) at room temperature.

Scanning electron microscopy

The morphology of powdered samples of atenolol, physical mixture, and inclusion complex were studied by scanning electron microscope. The conductive layer has been applied at the surface of the sample. The samples then were observed at 15 KV to obtain scanning electron images.

Preparation of atenolol stock solution and standard solution

The preparation of atenolol stock solution has been done by weighing 125 mg of atenolol accurately then dissolved in 25 ml of methanol in 25.0 ml volumetric flask. 10,0 ml of this solution was pipetted and transferred in 100.0 ml volumetric solution, then acetate buffer pH 4.6 was added to the volumetric flask until 100 ml. The concentration of atenolol stock solution was 500,0 ppm. The preparation of atenolol standard solution was done by diluting the stock solution of atenolol. The concentration of atenolol standard solution which has been prepared were 40, 50, 60, 80, 100, 125, and 200 ppm. The absorbance of the solutions were observed at λ 274 nm.

Linearity and range

Linearity and range of the standard solution were determined by observing the absorbance of the atenolol standard solution at 273.8 nm. Standard calibration curve of atenolol standard solution was produced by regression the concentration of standard solution vs absorbance. Linearity of the standard calibration curve can be determined by comparing the r value to the r table.

Specificity

Specificity of the analysis method was determined by observing the influence of β-cyclodextrin the absorbance of atenolol at λ 274 nm using UV spectrophotometer. The absorbance of 10 ppm β-cyclodextrin solution has been observed at λ 274 nm.

Drug content analysis

Inclusion complex powder was weight 133.4 mg (inclusion complex were equivalent to 25 mg of atenolol) were dissolved in 10 ml methanol. This solution then was transferred into volumetric flask 100.0 ml and a portion of acetate buffer pH 4.6 was added. The solution was sonicated thoroughly, then acetate buffer pH 4.6 was added to the volumetric flask until 100 ml. The filtrate was pipetted 10 ml and diluted with acetate buffer pH 4.6 ad 25 ml in a volumetric flask. Whatmann no.41 filter paper were used to remove the undissolved matter. This solution was assayed at λ 274 nm, using UV-Visible double beam spectrophotometer (Shimadzu UV-1800) (Chandrasekhar et al., 2013).

Dissolution study of atenolol-β-cyclodextrn inclusion complex

Dissolution profiles of inclusion complexes (equivalent to 25 mg of atenolol) was studied using the USP apparatus II (Paddle method) in Hanson and research® dissolution apparatus at 50 rpm for 60 minutes. Dissolution studies were carried out using 900 ml of acetate buffer pH 4.6 as dissolution medium. The dissolution medium was maintained at 37±0.5°C. The powder was poured into the chamber of dissolution apparatus. Aliquot (5 ml) was taken at specified time intervals (2, 4, 6, 8, 10, 15, 30, 45 and 60 minutes). The sample was replaced by an equal amount of fresh dissolution medium. The absorbance of the sample was analyzed using UV-visible, double beam spectrophotometer (Shimadzu UV-1800) at λ 274 nm. The atenolol concentration which was released to the media was calculated. The data presented is the average of three determinations. Dissolution profile for each formula was plotted and dissolution parameters such as %Q, tQ%, AUC, and dissolution efficiency was determined. Dissolution parameters (AUC and % ED) were analyzed statistically by one way ANOVA.

Preparation of powder mixture of orally disintegrating tablets of atenolol-β-cyclodextrin

Atenolol-β-cyclodextrin inclusion complex which was prepared using solvent evaporation method was mixed with several excipient to be compressed into orally disintegrating tablets. The composition of orally disintegrating tablets of atenolol can be seen

in table 1. Inclusion complex of atenolol with β-cyclodextrin was mixed with aerosil® for 3 minutes, then this mixture was mixed with a half portion of Avicel® PH 102. In the next step. This mixture was mixed with the remained of Avicel® PH 102, crospovidone, aspartam, manitol DC, and mint flavour for 10 minutes in tumbling mixer. The powder mixture then was evaluated before compression step (pre compression test). After pre compression test, the powder mixture was mixed with talk, magnesium stearate, and Aerosil 200® for 3 minutes. The powder mixture, then compressed into tablet using Erweka® tablet compression machine.

Pre compression evaluation

The powder mixture of orally disintegrating tablets of atenolol was evaluated by several parameters such as flowability, angle of repose, bulk density, tapped density, compressibility, Hausner ratio, and moisture content.

Flowability and angle of repose

Static method to determine angle of repose was used to predict the flowability of powder mixture. The powder mixture (± 100 gram) was poured through a funnel that can be raised vertically to a maximum cone height (h) was obtained (Kumare *et al.*, 2013). Angle of repose (Θ) of the physical mixture was determined by measuring the the radius of the heap (r) and the height of the cone. Angle of repose (Θ) was calculated using the formula:

$$\tan \theta = \frac{h}{r}$$

Time for the powder mixture to fall down through a funnel was used to calculate flowability of the powder.

Bulk density

The powder was poured into a graduated cyclinder. The bulk volume (Vb) and weight of the blend (m) was determined. The bulk density was calculated by this equation (Chandrasekar *et al.*, 2013).

$$\textbf{Bulk density} = \frac{\textbf{m}}{\textbf{Vb}}$$

Where,

m = the mass of powder mixture
Vb = bulk volume of the powder

Tapped density

Tapped density of the powder mixture was determined using tapping machine. Tapped density od the powder were calculated through the ratio of total mass of the powder (m) to the tapped volume of the powder (Vt). Volume was measured by tapping the powder for 500 times. The volume was read every 100 interval (Shirshand *et al.*, 2010). Tapped volume was noted, if the volume did not show a difference between two tapping intervals.

$$\textbf{Tapped density} = \frac{\textbf{m}}{\textbf{Vt}}$$

Where,

m = the mass of powder mixture
Vt = tapped volume of the powder

Compressibility

Compressibility index determination was carried out to evaluate compressibility of the powder and flow property. Compressibility index can be calculated by comparing the bulk density (Db) and tapped density (Dt) of the powder (Aulton and Summers, 2013).

$$\textbf{Compressibility index} = \frac{\textbf{(Dt} - \textbf{Db)}}{\textbf{Dt}} \textbf{ x 100}$$

Where,

Dt is the tapped density of the powder
Db is the bulk density of the powder

Hausner ratio

Hausner ratio is an indirect method to predict powder flow (Aulton and Summers,

2013). Hausner ratio can be calculated by following formula:

$$\textbf{Hausner ratio} = \frac{\textbf{Dt}}{\textbf{Db}}$$

Where,

Dt is the tapped density of the powder
Db is the bulk density of the powder

Moisture content

The moisture content of the powder was determined by analyzing approximately 5 grams of the powder. This evaluation was done by using moisture content analyzer. The moisture content of the powder can be calculated using this equation:

$$\%\, \boldsymbol{MC} = \frac{\boldsymbol{W-Wo}}{\boldsymbol{Wo}} \textbf{ x } \textbf{100\%}$$

Where,

W is the weight of wet mass
Wo is the weight of dry mass

Preparation of atenolol orally disintegrating tablets

The powder mixture was prepared for the compression process after pre compression evaluation. Tableting process was conducted by compress the powder mixture using the Erweka® tablet compression machine. The powder was compressed into 300 mg tablet using 11 mm flat punches.

Post compression evaluation

Orally disintegrating tablets of atenolol-β-cyclodextrin were evaluated through several parameters such as organoleptic, dimension, hardness, friability, wetting time, water absorption ratio, in vitro dispersion time, disintegration, drug content, and dissolution.

Organoleptic

The orally disintegrating tablets were inspected in several parameters such as color, shape, and taste.

Dimension

Orally disintegrating tablets were evaluated using vernier caliper to determine the thickness and diameter. 10 tablets of each formula were determined its thickness and diameter to ensure uniformity of tablet size.

Hardness

The hardness of orally disintegrating tablets were evaluated using a Monsanto hardness tester. The hardness of 10 tablets were determined using Monsanto hardness tester. The tablet was placed between the two jaws of the tester held along its oblong axis. In the initial step, the reading of the scale should be zero kg/cm^2. Constant force was applied by rotating the knob until the tablet fractured. The value which was displayed in the scale at this point must be noted (Chandrasekhar et al., 2013).

Friability

Weighed amount of dedusted tablets equal to 6.5 gram were subjected to rotating drum of Erweka rolling and impact durability tester. The drum revolves at speed of 25 rpm for 4 minutes (Lachman et al., 1991). The tablets were then dusted and reweighed. Percent weight loss (% friability) was calculated using the following equation:

$$\%\text{Friability} = \frac{W_1 - W_2}{W_1} \times 100$$

Where W_1 was the weight of the tablet before test and W_2 was the weight of the tablet after test.

Wetting time and water absorption ratio

Wetting time test was carried out to described the inner structure of the tablets and hydrophilicity of the excipients. Small petri plate was prepared with 10 ml eosin. A piece of a

tissue paper folded twice was placed in a small petri. Orally disintegrating tablet of atenolol-β-cyclodextrin was placed carefully on the surface of filter paper. The time of eosin solution to cover the surface of the tablet was noted (Kumare *et al.*, 2013). The same procedure was carried out to predict the ability of the tablet to absorp water. Water absorption ratio (R) was determined using this equation:

$$R = \frac{Wa-Wb}{Wb} \times 100$$

Where Wb and Wa were tablet weight before and after absorption

In vitro dispersion time

A flask containing 10 ml phosphate buffer pH 6.8 with temperature 37±0.5°C was prepared. Orally disintegrating tablet was placed in this flask. The time required for complete dispersion was determined. This study was done by three times replication (Nalkwade et al., 2013).

Disintegration time

Orally disintegrating tablet was placed in the disintegration time apparatus. The tablet was placed in each of the six tubes. The apparatus was operated using 900 ml distilled water maintained at 37±0.5°C as the immersion fluid. The time for complete disintegration of the tablet with no palpable mass replaced on the screen was measured in seconds (Shivanand et al., 2010). The results revealed the disintegration time of the tablets.

Drug content

Drug content in orally disintegrating tablet of atenolol-β-cyclodextrin was done by randomly sampling 20 tablets from each formula, and then these tablets were weighed and triturated in a mortar. The powder equivalent to 25 mg atenolol was weighed accurately and dissolved in 10 ml methanol. The solution transferred to 100 ml volumetric flask and a portion of acetate buffer pH 4.6 was added. The solution was sonicated thoroughly, then acetate buffer

pH 4.6 was added to the volumetric flask until 100 ml. Whatmann no.41 filter paper was used to remove the undissolved matter. The filtrate was pipetted 10 ml and diluted with acetate buffer pH 4.6 ad 25 ml in a volumetric flask. This solution was assayed at λ 274 nm, using UV-Visible double beam spectrophotometer (Shimadzu UV-1800) (Chandrasekhar et al., 2013).

Dissolution study

Dissolution parameter of orally disintegrating atenolol-β-cyclodextrin was studied using USP dissolution apparatus II (paddle) at 50 rpm for 120 minutes. Acetate buffer pH 4.6 was used as a dissolution medium which was maintained at 37±0.5°C. Aliquot (10 ml) was taken at specified time intervals. Fresh dissolution medium was prepared and replaced immediately to accomodate the withdrawal of the sample. The samples were filtered through syringe filter (0.45 µm). Solution of Samples were analyzed using UV-visible, double beam spectrophotometer (Shimadzu UV-1800) at λ 274 nm. The dissolution parameters such as %Q, tQ%, AUC, and dissolution efficiency were determined (United States Pharmacopeial Convention, 2017).

RESULT AND DISCUSSION

Characterization of atenolol-β-cyclodextrin inclusion complex

Inclusion complexes were prepared using different methods, such as physical mixture, kneading method, and solvent evaporation. The organoleptic of inclusion complex were found to be white powders and no odor.

Fourier transform infrared spectrophotometry

The interaction and complex formation between drug molecules and β-cyclodextrin in the solid state were analyzed by Fourier transform infrared spectrophotometry is a useful technique to assess. Significant changes in the shape and position of absorbance bands revealed the complex formation between drug molecules and β-cyclodextrin. This step was carried out

to predict the interaction of atenolol and β-cyclodextrin in 1:1 ratio. Infrared spectrum of atenolol, β-cyclodextrin, physical mixture of atenolol-β-cyclodextrin, and an inclusion complex of atenolol- β-cyclodextrin can be seen in figure 1.

Infrared spectrum of pure atenolol showed bands in 3354,57 cm^{-1} and 3173,29 cm^{-1} indicated -CO-NH group, 2964,05 cm^{-1} (=CH), 1636,03 cm^{-1} (-C=O, NH primer), and 1515,78 cm^{-1} (-N-C=O, NH secondary). Atenolol molecules had a carbonyl band of 1725-1685 cm^{-1}. Infrared spectrum of physical mixture showed no significant change of this band. Whereas, infrared spectrum of inclusion complex using kneading method and solvent evaporation method showed a change in carbonyl band and a significant decrease was observed in its intensity. These phenomenon can be caused by the dissociation of the intermolecular hydrogen bonds of the atenolol through inclusion complex. This condition may have resulted from atenolol restriction within β-cyclodextrin cavity (Ficarra et al., 2000). Infrared spectrum of physical mixture of atenolol- β-cyclodextrin showed that one functional group (-CO-NH) disappeared . Moreover, in inclusion complexes which had been prepared by kneading and solvent evaporation method, two functional groups (-CO-NH) had been bounded by functional OH groups of β-cyclodextrin through hydrogen bonding (Kumar et al., 2014). The comparison of specific groups of infrared spectrum among atenolol, β-cylcodextrin, and inclusion complex were presented in table 3.

The spectrum of drug showed a sharp peak at 3354 cm^{-1}. This peak was broaden in inclusion complex. This phenomenon can be seen by shift of this peak in the inclusion complex spectrum. Moreover, peak in wavelength 3174 cm^{-1}, originating from the NH valence vibrations indicating the difference pattern between atenolol and its inclusion complex. NH peak was present in drug spectra. This peak was totally absent in the spectra of the inclusion complex because of host guest complex formation with β-cyclodextrin (Nikolic et al., 2007; Gite et al., 2014).

Differential scanning calorimetry (DSC)

Thermal behavior of atenolol, physical mixture of atenolol-β-cyclodextrin, and inclusion complex of atenolol-β-cyclodextrin was studied in order to analyze the complex formation. The DSC thermogram of atenolol, physical mixture of atenolol-β-cyclodextrin, and inclusion complex of atenolol-β-cyclodextrin are shown in figure 2. Endothermic peak was found in the DSC thermogram of atenolol. The endothermic peak at 153.50°C revealed its melting point. The β-cyclodextrin showed broad endothermic peak, which is approximately located in 122.43°C (nearer to 100) due to release of water molecules. The physical mixture and inclusion complex which was prepared using kneading method showed an endothermic peak. Physical mixture showed two endothermic peaks at 120,89°C and 151,46°C. Whereas, inclusion complex which was prepared by kneading method showed two endothermic peak at 121.27°C and 149.71°C. Inclusion complex of atenolol with β-cyclodextrin showed one endothermic peak at 146.68 °C. The complete disapearance of atenolol indicates that there is formation complex (Gite et al., 2014).

Analysis of crystallographic aspect using powder X-ray diffraction (XRD)

The inclusion complex of atenolol with β-cyclodextrin, physical mixture, and pure atenolol were characterized further by an X-ray diffraction (XRD) study. The X-ray diffraction patterns of pure atenolol, as well as the atenolol- β-cyclodextrin inclusion complexes obtained by using kneading method and solvent evaporation method are represented in figure 3. Crystal structure of inclusion complex and atenolol was determined using analysis of crystallographic. Inclusion complex formation can be analysed using X-ray diffraction. Inclusion complex of atenolol-β-cyclodextrin were determined by appearence of new or at least deviation from the original pattern. X-ray diffraction pattern of atenolol and β-cyclodextrin indicated that they are present in crystalline form. X-ray diffraction pattern of physical mixture confirmed by the

presence of the peaks of both components of the mixture.

The comparison of X-ray peaks from the sample were tabulated in table 4. The results showed that there was the decrease of peak intensity of atenolol in inclusion complex. The highest reduction of peak intensity of atenolol was shown by inclusion complex, which was prepared using solvent evaporation method. In case of atenolol complexed with β-cyclodextrin diffractogram, it caused by a new solid phase with low crystallinity, indicating inclusion complex formation. The formation of the amorphous states indicated that atenolol formed complex with β-cyclodextrin. It showed that some molecule of atenolol incorporated in the cavity of β-cyclodextrin.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) images of atenolol, the physical mixture, inclusion complex (prepared by kneading method and solvent evaporation method were studied. SEM study showed the morphology and microscopy photography of the drug and its inclusion complex. The representative images are shown in figure 4. The shape of pure drug particles were irregular. The physical mixture images showed that only a small amount of atenolol which were attached on the surface of β-cyclodextrin. The higher amount of atenolol particles attached on the surface of β-cyclodextrin in inclusion complex which were produced by kneading method. Moreover, the image of inclusion complex which are produced by solvent evaporation method showed that atenolol particles attached and incorporated into β-cyclodextrin particles. The structure of atenolol in inclusion complex, which was produced by solvent evaporation method became more amorphous. The solvent evaporation inclusion complex was poor of crystal structure, lack distinct crystal faces, and incorporated completely in β-cyclodextrin structure. This phenomenon also contribute to the faster dissolution of atenolol compared to the inclusion complex which were prepared using kneading method (Ghosh et al., 2011).

Linearity

The concentration of atenolol standard solution (40.0 ppm – 200.0 ppm) were plottted versus absorbance to obtain atenolol calibration curve. The regression equation was y = 0.0037 + 0.0045X. Coefficient correlation between atenolol concentration in standard solution and absorbance was calculated. The coeficient correlation was found 0.9999. This results indicated which the calibration curve was linear.

Specificity

Specificity of the analysis method was determined by observing the influence of β-cyclodextrin the absorbance of atenolol at λ 274 nm using UV spectrophotometer. The solution of β-cyclodextrin 10.0 ppm did not show absorbance at λ 274 nm. This result indicated that β-cyclodextrin did not affect the absorbance results of atenolol at λ 274 nm.

Drug content

UV spectrophotometry was used to analyzed the drug content of the inclusion complex of atenolol-β-cyclodextrin. The results showed that the drug content of physical mixture was 91.47±1.22%, the inclusion complex which was produced by kneading method was 92.59±0.90%, and the inclusion complex which was produced by solvent evaporation method was 95.40%±0.97.

Dissolution study of inclusion complex

Dissolution profile of physical mixture, inclusion complex which was produced by kneading method, and inclusion complex which was produced by solvent evaporation method are shown in figure 5 and table 5. Inclusion complex, which was prepared using solvent evaporation method showed the highest percentage of drug release in 30 minutes and dissolution efficiency (%ED). The dissolution profiles of physical mixture and inclusion complexes showed that the solvent evaporation complex exhibited a faster dissolution rate than the physical mixture and

the inclusion complex which was produced by kneading method. Dissolution parameters of the physical mixture and inclusion complexes as shown in table 2. Analysis using one-way ANOVA revealeed that there was a significant difference of dissolution among atenolol-β-cyclodextrin physical mixture, inclusion complex using kneading method, and inclusion complex using solvent evaporation method. Inclusion complex of atenolol-β-cyclodextrin produced the highest AUC and dissolution eficiency compare to the physical mixture and kneading method. Based on the previoud results, it can be concluded that solvent evaporation method produces the best characteristics of atenolol and β-cyclodextrin. The inclusion complex, which was produced by solvent evaporation method was continued to develop into orally disintegrating tablets.

Pre compression evaluation

The flow properties of powder mixture must be analyzed to predict the uniformity of the powder misxtue (mass of the tablets). The homogeneity of powder flow will produce a low variation of tablet weight (Hahm and Augsburer, 2008). The flow velocity of the powder blend also must be determined to predict the ability of powder blend to fulfill the dies during compression stage. The flow properties of powder mixture were analyzed before compression of the tablets. Compressibility index (%) and Hausner ratio were calculated to determine the flow properties (United States Pharmacopeial Convention, 2017). The results of flow velocity and angle of repose were found that the powder can not flow well in a glass funnel. This was due to the high percentage of fines in powder mixture. The powder mixture which had a high percentage of fines was more adhesive or cohesive. The flow of the powder in this situation was not influenced by gravitation force (Aulton and summer, 2013). The results of compressibility index and Hausner ratio revealed that the powder mixture had poor flow

character (United States Pharmacopeial Convention, 2017). This problem can be solved by decreasing the fines percentage and controlling the particle size distribution (Aulton and summer, 2013).

Moisture content evaluation of powder mixture was conducted to determine that the products had high mositure content or not. The results showed that the powder mixture had high moisture content (6.21%±0,.23). The high moisture content of powder mixture probably caused by the excipient which were hygroscopic, such as β-siklodekstrin, *crospovidone,* and Avicel® PH 102. The powder mixture which had a high humidity, will be more cohesive so that this mixture did not flow well. Therefore the weighing process, the mixing process, and tabletting process must be conducted in a room which the humidity and temperature are controlled well.

Post compression evaluation

Physichochemical characteristics of atenolol orally disintegrating tablets are tabulated in table 2. Atenolol orally disintegrating tablets were white, round shape, no odor, sweet and mint flavor. The uniformity of tablet size could be evaluated by measuring the tablet mean thickness and diameter. The thickness was approximately 4.00 mm, revealed that the size of the tablets were uniform. The prepared tablets in all the formulations possessed good mechanical strength. It can be concluded based on the results of hardness test. The hardness of orally disintegrating tablets of atenolol-β-cyclodextrin was 2.49 ± 0.41 kg. The specification of tablet hardness in orally disintegrating tablets are 2.0-4.0 kg (Hahm and Augsburger, 2008). Friability and abrasion values of orally disintegrating tablets of atenolol were below 1%. This result indicating that orally disintegrating tablets of atenolol-cyclodextrin have good mechanical resistance.

The wetting time for orally disintegrating tablets of atenolol-β-cyclodextrin was 124.67±3.79 seconds. The faster wetting time of the tablets, the tablets will be disintegrated rapidly when contact with media. Water absorption ratio test was conducted to predict the amount of water which can be absorbed by orally disintegrating tablets. Orally disintegrating tablets which have lower water absorption ratio were more preferable to develop. This was due to the orally disintegrating tablets only need a small amount of water to disperse in the media (Panigrahi *et al.*, 2010).

In vitro dispersion time was conducted to predict the ability of orally disintegrating tablets to be dispersed in a small amount of saliva in the oral cavity. Orally disintegrating tablets of atenolol-β-cyclodextrins dispersed in 45.,33 ± 0.58 seconds. The disintegration time of atenolol-β-cyclodextrins tablets were 8.17 ± 0.41 seconds. These results showed that orally disintegrating tablets of atenolol-β-cyclodextrins met the specification which has been stated in compendia (<1 minutes) (US Departement of Health and Human Services, 2008). The faster disintegration of the tablets caused rapid capillary activity and pronounced hydration with little tendency to gel formation (Kulkarni et al., 2011).

Dissolution profile of orally disintegrating tablets of atenolol- β-cyclodextrin showed in figure 6. The results from the dissolution study showed that orally disintegrating tablets atenolol-β-cyclodextrin meet the specification of compendia. The specification stated that the minimum amount of drug dissolved in 30 minutes is 85%. The amount of drug dissolved from this formula was 92.22%, so it can be concluded that this orally disintegrating tablets met the specification.

CONCLUSION

The results from this study showed that solvent evaporation method produces the best physichochemical characteristics of inclusion complex of atenolol-β-cyclodextrin.

Consequently, solvent evaporation method was chosen to produce inclusion complex of atenolol-β-cyclodextrin which further developed into orally disintegrating tablets. Orally disintegrating tablets of atenolol-β-cyclodextrin with sufficient mechanical strength, fast dispersion and disintegration time, and acceptable taste were produced from this study.

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