

# Artikel 8

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## Original Article

# Influence of Milling Process on Efavirenz Solubility

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### ABSTRACT

2 **Introduction:** The aim of this study was to investigate the influence of the milling process on the solubility of efavirenz. **Materials and Methods:** Milling process<sup>26</sup> was done using Nanomilling for 30, 60, and 180 min. Intact and milled efavirenz were characterized by powder X-ray diffraction, scanning electron microscopy (SEM), spectroscopy infrared (IR), differential scanning calorimetry (DSC), and solubility test. **Results:** The X-ray diffractogram showed a decline on peak intensity of milled efavirenz compared to intact efavirenz. The SEM graph depicted the change from crystalline to amorphous habit after milling process. The IR spectrum showed there was no difference between intact and milled efavirenz. Thermal analysis which performed by DSC showed a reduction on endothermic peak after milling process which related to decreasing of crystallinity. Solubility test of intact and milled efavirenz was conducted<sup>13</sup> in distilled water free CO<sub>2</sub> with 0.25% sodium lauryl sulfate media and measured using high-performance liquid chromatography method with acetonitrile: distilled water (80:20) as mobile phases. The solubility was significantly increased ( $P < 0.05$ ) after milling processes, which the intact efavirenz was  $27.12 \pm 2.05$ , while the milled efavirenz for 30, 60, and 180 min were  $75.53 \pm 1.59$ ,  $82.34 \pm 1.23$ , and  $104.75 \pm 0.96$   $\mu\text{g/mL}$ , respectively. **Conclusions:** Based on the results, the solubility of efavirenz improved after milling process.

**KEYWORDS:** Crystallinity, efavirenz, milling, solubility

## INTRODUCTION

10 It is widely known that the active pharmaceutical ingredient in a solid dosage form must be dissolved before it is available for absorption from the gastrointestinal tract. Most drug compounds have low bioavailability due to low solubility and dissolution rate. Plasma drug concentration will affect the therapeutic effectiveness of<sup>28</sup> drug compound.<sup>[1]</sup> The solubility of drug molecules is one of the most challenging aspects in the formulation development. Successful formulations depend on how to produce active pharmaceutical ingredients exist on the active site which contributed to the pharmacological effect, efficiently.<sup>[2]</sup>

32 A large number of approaches to improve the solubility of poorly soluble drugs have been developed. These strategies include methods for particle size reduction, the formation of amorphous phase, solid dispersion system, and cocrystal phase.<sup>[3-5]</sup> Milling is a major process in the preparation of various forms of solid state, particularly at the last stage in the production where the size of the

drugs particles decreased and also accompanied by a decrease in crystallinity due to the occurrence of crystal defects. Crystal defects generated by milling may cause phase transformation and finally amorphization. An amorphous solid is more soluble than the crystalline phase.<sup>[6,7]</sup>

1 Efavirenz is a nonnucleoside reverse transcriptase inhibitors class of antiretroviral which specific to HIV Type 1. Efavirenz is also classified in Class II according to the biopharmaceutical classification system, which has high membrane permeability but has low solubility. Drugs with high permeability and low solubility often show a low gastrointestinal absorption due to poor solubility of the drug in gastrointestinal fluids which will lead to low oral bioavailability as well.<sup>[8]</sup>

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The previous studies in increasing the solubility of efavirenz have been done by several methods such as formation of cocrystal phase, solid dispersion, and simple eutectic mixture.<sup>[8-10]</sup> Therefore, in this study, particle size reduction of efavirenz will be conducted by milling to see any improvement of its solubility.<sup>[25]</sup> The intact and milled efavirenz will be analyzed by powder X-ray diffraction (XRD) analysis, scanning electron microscope (SEM), spectrometry infrared (IR) analysis, differential scanning calorimetry (DSC) analysis, and solubility test.

## MATERIALS AND METHODS

### Materials

Efavirenz (Kimia Farma, Indonesia), acetonitrile high-performance liquid chromatography (HPLC) grade (Merck, Germany), methanol pro analysis (Merck, Germany), aquabidest (PT Ikapharmindo Putramas), methanol HPLC grade (Merck, Germany), and sodium lauryl sulfate (SLS) (Brataco, Indonesia).

### Preparation of milled efavirenz

As much as 7 g of Efavirenz was milled at 500 rpm using Nanomilling (Fritsch, Pulverisette 7, Germany) for 30, 60, and 180 min. The milled efavirenz was then kept in desiccator.

### X-ray powder diffraction analysis

Analysis of the X-ray powder diffraction was done using a diffractometer (X'Pert XRD Powder type PW 30/40 PANalytical, The Netherlands). The sample was placed on sample holder and leveled to prevent particle orientation during sample preparation. The measurement was done at conditions as follows: the target metals Cu, filter K $\alpha$ , 45 kV voltage, 40 mA current, the analysis carried out in the range of 2 theta 10°–40° at room temperature. The analysis was carried out for unmilled, and 30, 60, and 180 min milled efavirenz.

### Scanning electron microscopy analysis

Sample powder was placed on the sample holder aluminum and coated with gold. The sample was observed at various magnifications SEM (Jeol type JSM-6360 LA, Japan) with the voltage was set at 20 kV, and the current was 12 mA. SEM analysis was conducted for intact efavirenz and milled efavirenz for 180 min.

### Fourier transform infrared spectroscopy analysis

Samples were analyzed using an IR spectrophotometer (PerkinElmer Fourier transform IR [FT-IR] Spectrophotometer, The United States of America) by dispersing samples on KBr plate and were compressed at high pressure (hydraulic press). Absorption spectra were recorded with (FT-IR) at wavenumber 600–4000 cm<sup>-1</sup>. The analysis was carried

out for unprocessed and milled efavirenz for 30, 60, and 180 min.

### Differential scanning calorimetry analysis

Thermal analysis was carried out using DSC (Mettler Toledo FP90, Switzerland) which its temperature has been calibrated with indium. A small amount sample (about 6.5 mg) was placed on an aluminum pan. DSC temperature range was programmed at 30°C up to 250°C with a heating rate 10°C/min. The analysis was conducted for intact efavirenz and milled efavirenz for 180 min.

### Solubility test

Solubility test was conducted on intact efavirenz and milled efavirenz for 30, 60, and 180 min. The sample was made into a saturated solution. An excessive amount of the samples were added to 100 ml of distilled water free of CO<sub>2</sub> with 0.25% SLS. Test was conducted for 24 h using an orbital shaker. Samples were filtered using Whatman filter paper (0.45  $\mu$ m), and 1 mL of filtrate solution was then taken, and levels of efavirenz dissolved were measured by HPLC with a mobile phase of acetonitrile: distilled water (80:20). The HPLC method was developed by experiments.

### Statistical analysis

The result of solubility tests of intact efavirenz, 1, 2, and 3 h milled efavirenz was analyzed statistically using *post hoc* P values for the Duncan test of one-way analysis of variance.

## RESULTS AND DISCUSSION

### Powder X-ray diffraction

X-ray diffraction is a very useful method for determining the presence of an amorphous phase, polymorphism, or modification of crystal habit in solid drugs. The

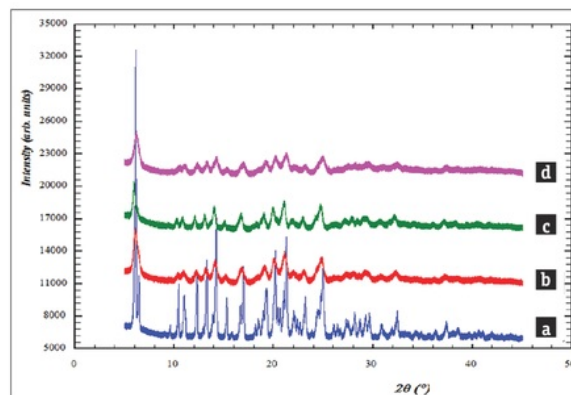


Figure 1: Powder X-ray diffraction pattern of (a) intact efavirenz, (b) milled efavirenz 30 min, (c) milled efavirenz 60 min, and (d) milled efavirenz 180 min

diffractogram of intact and milled efavirenz for 30, 60, and 180 min can be seen Figure 1.

The peak position of intact efavirenz and milled efavirenz for 30, 60, and 180 min at  $2\theta$  did not change, which indicated that no polymorphic transformation of efavirenz occurred after milling process. Provision of mechanical energy by milling for 30 min, 1 h, and 3 h did not change the crystal lattice of efavirenz. However, the diffractogram showed the intensity peak of efavirenz declined after milling process. The crystallinity of milled efavirenz for 180 min was lower than milled efavirenz for 30 and 60 min. This likely due to that milling process caused partially transformation from crystalline into amorphous phase which was indicated by the decreasing in crystallinity of efavirenz.<sup>[3]</sup>

#### Scanning electron microscopy analysis

SEM of intact efavirenz and milled efavirenz for 180 min can be seen in Figure 2. The intact efavirenz at 1000 times magnification depicted a solid rod-shaped crystalline, while milled efavirenz for 180 min at same magnification showed a deformation from crystal into irregular (amorphous) shape. This showed that the powder milling results caused transformation into amorphous phase by reducing the degree of crystallinity which in accordance to result the X-ray diffraction.

#### Fourier transform infrared spectroscopy

IR spectroscopic analysis was conducted to identify the functional groups of a compound. The IR spectroscopy characterization of intact efavirenz and milled efavirenz can be seen in Figure 3. There was no change observed between intact efavirenz functional groups and milled efavirenz, which indicated that no changes occur in the compound of the crystal after milling process.

#### Differential scanning calorimetric analysis

DSC is a method to characterize the thermodynamic properties that occur at the time the samples were subjected to heat energy, which is indicated by an endothermic or exothermic peak in the DSC thermogram.<sup>[11]</sup> The result of thermal analysis of intact efavirenz and milled efavirenz for 180 min can be seen in

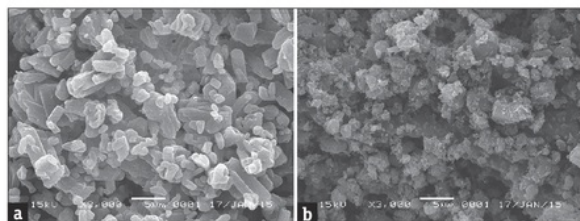


Figure 2: Scanning electron microscopy of (a) intact efavirenz, (b) milled efavirenz

Figure 4. Efavirenz has an endothermic peak at 135.7°C which requires the heat of 47.5 kJ/g, while milled efavirenz has 136.4°C endothermic peak and requires heat of 43.42 kJ/g. This decreasing in enthalpy indicated a reduction of crystallinity due to milling process, therefore, the heat energy required to melt efavirenz was lower for milled efavirenz compared to intact efavirenz.<sup>[6]</sup>

#### Solubility test

Solubility is one of the physicochemical properties of drug compounds that can predict the degree of absorption of the drug in the gastrointestinal tract. The solubility of efavirenz after milling process increased significantly ( $P < 0.05$ ), which can be seen in Table 1. From these results, it can be concluded that an increase in the solubility between intact efavirenz with efavirenz after milling was supported by the results of X-ray diffraction that showed a decline in the degree of crystallinity.<sup>[7]</sup>

#### CONCLUSIONS

Milling process could alter the crystallinity degree of efavirenz which transforms the crystalline form to partial

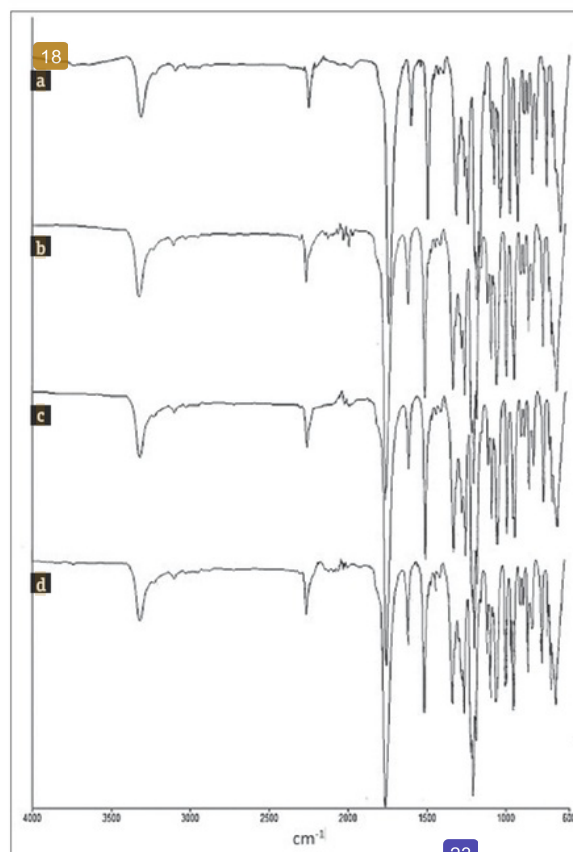


Figure 3: Infrared spectrum of (a) intact efavirenz, (b) 30 min milled efavirenz, (c) 60 min milled efavirenz, and (d) 90 min milled efavirenz

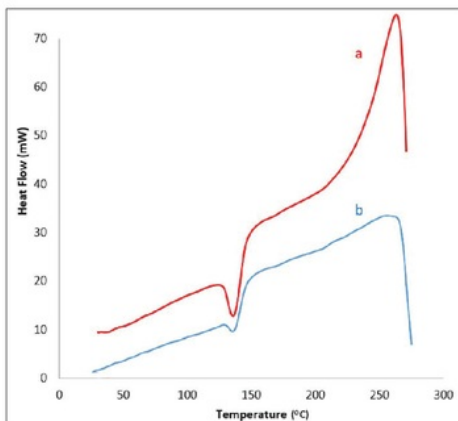


Figure 4: Thermogram of (a) intact efavirenz, (b) milled efavirenz

Table 1: Solubility of intact and milled efavirenz

Materials	Solubility ( $\mu\text{g/mL}$ )
Intact efavirenz	27.11 <sup>a</sup> ±2.04
30 min milled efavirenz	75.53 <sup>b</sup> ±1.59
60 min milled efavirenz	82.34 <sup>c</sup> ±1.23
17 min milled efavirenz	104.75 <sup>d</sup> ±0.96

Means followed by the same letter in the column do not differ statistically at 0.05 probability level

amorphous phase and contributed to increasing the solubility. The decreasing of crystallinity affects thermal behavior, where the endothermic and heat energy of efavirenz reduced after milling process.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

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