Polymorphic properties and dissolution profile of efavirenz due to solvents recrystallization

Yoga Windhu Wardhana1,2*, Sundani N Soewandhi1, Saleh Wikarsa1 and Veinardi Suendo3

1Department of Pharmaceutics, School of Pharmacy, Institute Technology of Bandung, Bandung, Indonesia
2Department of Pharmaceutics and Pharmaceuticals Technology, Faculty of Pharmacy, University of Padjadjaran, Bandung, Indonesia
3Inorganic and Physical Chemistry Division, Faculty of Mathematics and Natural Sciences, Institute Technology of Bandung, Bandung, Indonesia

Abstract: Polymorphism occurs in pharmaceutical compounds affect to the physicochemical quality and goal of therapy. Thus, quality evaluation of different crystal forms should be assessed especially the solubility and dissolution behaviors among polymorphic forms, which correlate to bioavailability and therapy efficacy. To achieved the different of a polymorph various solvent were used such as acetonitrile, methanol, ethyl acetate, acetone, water, n-hexane, and n-heptane. All of the crystal modification resulted were characterized by a polarization light microscopy (PLM), Fourier-Transform Infrared (FTIR) differentia l scanning calorimetry (DSC) and powdered X-ray diffraction (PXRD). Besides that, nature of solubility in water (24 and 48 hours test times) and particulate dissolution profile (an hour test) were carried out. There were various polymorphs success resulted and have significant differences in morphology, definite spectral fingerprints, crystal structure and thermal behavior. From the solubility of the samples found the top three highest soluble forms i.e. Form 6, 2 and 3, respectively. But there are showed became in order reverse performance after 60 minutes dissolution (Form 3, 2 and 6, respectively). The polymorphic forms of EFV were successful to obtained by the solvents treatment. Therefore, the physicochemical properties of polymorphic forms from active pharmaceutical ingredients (APIs) should be carefully considered in dosage forms pre-formulation approaches.

Keywords: Efavirenz, polymorphism, polymorph properties, solubility, dissolution profile.

INTRODUCTION

The polymorphism phenomenon among active pharmaceutical ingredients (APIs) has been concerned a lot in this decade. Polymorphism, which API in the chemically similar formula can exhibit than one crystalline structure resulted in differences nature of polymorphs (Braga, 2009). The nature of the polymorphs will affect the physicochemical quality of dosage forms, especially the bioavailability and therapy efficacy (Brittain, 1999; Hilfiker, 2006; Sarma 2011). So, these changeable behaviors among polymorphs in raw materials should be evaluated carefully.

There are several factors to modified crystal of APIs such as supersaturation, agitation rate, cooling rate, solvent composition, temperature, seed crystals, additives, and impurities. Among these factors, the solvent effect has studied dominantly (Milosovich, 2006; Mangin 2009; Chadha, 2011; Tran, 2012). Hence, the solvent selection in pharmaceuticals manufacturing became foremost importance consideration to keen specified product quantity.

One of API sample with a lot of polymorphic forms is efavirenz. At initially United States Food Drug Administration (US FDA) approved in 1998 haven’t known any polymorphic form. Recently, efavirenz (EFV) ((S)-6-chloro-4-(cyclopropyl ethynyl)-1,4-dihydro-4-(trifluoro-methyl)-2H-3,1-benzoazin-2-one) as the second generation of non-nucleoside reverse transcriptase inhibitor (NNRTI) for antiretroviral HIV–1 therapy has reported for 23 different forms include amorphous (Radesca et al., 1999, 2004; Sharma et al., 2006; Khanduri et al., 2006; Reddy et al., 2006; Doney, 2007; Dova, 2008; Ravikumar 2009; Chadha, 2011). But, these publications and patents have to describe their characterization are obscure and incomplete information. In addition, this study as part of EFV raw material screening assays for application strategies in dosage forms.

This study aims to investigate the different solvents influences on the physicochemical behaviors among the polymorphic forms resulted. To obtain this purpose, EFV was recrystallized by various solvents (acetonitrile, methanol, n-Hexane, and n- Heptane) and some mixed solvents (methanol-ethylacetate and acetone-water) as well. Polarization Light Microscopy (PLM), Fourier Transform Infrared (FTIR), Differential Scanning Calorimetry (DSC), Powdered X-ray Diffractometry (PXRD) were used to identify the crystalline structure resulted. Besides that, the solubility and dissolution profile of polymorph were carried out.

*Corresponding author: e-mail: ayodhna@gmail.com
MATERIALS AND METHODS

Materials
A pharmaceutical grade of Efavirenz (EFV, Batch No. EZ1670711, Hetero Labs. Ltd., India) was purchased from PT. Kimia Farma Tbk, Indonesia. The analytical grade of solvents such as acetonitrile, acetone, ethyl acetate, n-Hexane, n-Heptane, and methanol (ex-Merck, USA) was used in this work. All solvents used were of analytical reagent grade without further purification.

Methods
Recrystallization to obtain polymorphs
The various polymorph forms were made by recrystallization from each of the following solvents of analytical grade (came along with volumes and temperatures during super-saturation): acetonitrile (50 mL, 75°C), n-He-xane (100mL, 70°C), methanol (100 mL, 60°C), and n-Heptane (100mL, 90°C). All supersaturated solutions were filtered and kept at room temperature (25–28°C) for slow recrystallization. The solid phase came after few days from a solution of acetonitrile (20 days); n-Hexane and methanol (10 days), and n-Heptane (7 days). Collect those powder in a different container and labeled each as Form 1, Form 2, Form 3 and Form 5, respectively.

 Whereas for mixed solvents used by different methods, for methanol-ethyl acetate was made by mixture EFV with 2 mL ethyl acetate and 8 mL methanol at room temperature (25°C). The super-saturated solution was filtered and kept at room temperature (25-28°C) for slow recrystallization. Two days later solid phase was found, collect it and labeled as Form 4. Meanwhile for acetonitrile-water was made by mixture EFV with 5 mL acetone at room temperature (25°C) then moving the supersaturation solution to the ice container and added about 10 mL of ice (frozen aquadest at 0-5°C), let it still for 30 minutes. After that, stirred up for an hour and filtered with a vacuum. The supernatant resulted was drying in an oven at 55-60°C and kept for 5 hours. The dried powder stored and labeled as Form 6.

Polarization light microscope
Those crystals morphology were observed using an Olympus BX53 model U-LH100-3 microscope. The sample was placed on a microscope slide and covered with a cover-slip.

Fourier transform infrared (FTIR)
Fingerprints of the sample were recorded on a multi-scope spectrophotometer (IR Prestige-21 Shimadzu, Japan) by sealing the sample between two KBr plates by a hydraulic press under 200 kg/cm² for 15 seconds to form a disc. The spectrum for each sample was analyzed in the range 300-4500 cm⁻¹ spectral region with a resolution of 4 cm⁻¹.

Differential scanning calorimetry (DSC)
The thermal profile of polymorphs produced was obtained using NETZSCH DSC 214 Polymer on aluminum crucible with about 1-3 mg of samples under a dynamic nitrogen atmosphere and a heating rate of 10°C/min in the temperature range from 30 to 250°C. Before used, the DSC device already calibrated with indium as a standard reference.

Powder X-Ray diffraction (PXRD)
The crystallinity of powders was investigated by an X-ray diffractometer (XPERT-PRO, PANalytical, Netherlands) with Cu-Kα radiation as tube anode. The diffractogram patterns were recorded under following conditions: voltage 40 kV, 30 mA and fixed divergence slit using the configuration; 2θ range: 5° to 45°, 0.02 step size, 0.8 s time per step, care was taken to avoid phase transformations during sample preparation.

Aqueous solubility studies
Approximately about of 10 mg samples was dissolved in 100 mL aquadest using mechanical agitator shaker at 120 rpm for 24 and 48 hours under room temperature. The sample solution was filtered using millipore 0.45 µm and suitably diluted prior to measured by spectroscopy UV at 248 nm.

Particulate dissolution studies
The dissolution rate of each polymorph was determined as specified in USP36-NF31. In that USP specifications state, sodium lauryl sulfate (SLS) as 1-2% w/v was used for EFV. But for biorelevant considered, the SLS concentration selected was 0.25% w/v (Fandaruff, 2014; Panikumar, 2012). The dissolution methods using apparatus type 2 at 50 rpm and at a temperature of 37±0.5°C in Sotax AG CH-4008 BASEL, type AT-6. Sample solutions withdrawal at definite intervals until 60 minutes point of the collection were filtered through a 0.45 µ Millipore filter. Collected samples were suitably diluted with dissolution medium and analyzed at 248 nm using double beam spectrophotometer UV–Vis.

Content calibration curve preparation
Standard solutions were made from 10 mg of untreated EFV which was dissolved in methanol and 0.25% w/v SLS solution (1:9 v/v) then added slowly aquadest till 100 mL. Dilute the standard solution with 5 different concentrations i.e. 5, 7, 9, 11 and 13 ppm. All of the concentrations were measured by double beam spectrophotometer UV–Vis (SPECORD 200, Analytic Jena) at 248 nm. Absorbance parameter obtained from each standard solutions were plotted to be calibration curve to determine regression linear equation for content calculation standard.
RESULTS

To prove that all solvents and recrystallization methods affect to a pharmaceutical compound in various molecular arrangements resulted in more different polymorphic forms some characterization carried out. Firstly proven that structure modification happens simply observe by different object shape visually. The morphology of habit alteration of crystal compound could sight by polarization light microscopy.

To comprehend what kind of functional groups respond to change the molecular arrangement Fourier Transform Infrared (FTIR) spectroscopy was implemented. From the spectral performance shows at fig. 2 that between 400-4500cm⁻¹ had any luminous different spectrum came insight.

Those polymorphic forms may apply in dosage forms later. But before it used, the properties of its stability and compatibility during manufacture should be studied well. Good manufacturing practice is the pick of optimal processing energy to keep in good quality. So, energy in real life presented by changing of heat (thermal). Thereby, thermal analysis is important to know the substance behavior under various energy (thermal) treatment during the process.

Polymorphic forms identification among internal structure was powerful distinguishing by PXRD. The diffractogram patterns show the position of the atoms in molecular structure arrangements. As looked in fig. 4. Those crystals came from recrystallization were different pattern appearance.

As known in theory that polymorphic forms involve to solubility and its dissolution properties. So, to known is
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There any correlation between solubility and its dissolution profile, then some polymorphs as the top 3 (Form 6, 2 and 3, respectively) highest was compared with the stable one (Form 1) in particulate dissolution test, as shown in fig. 5.

Table 1: Solubility properties of EFV polymorphs

<table>
<thead>
<tr>
<th>Samples</th>
<th>Solubility Properties (µg/mL)</th>
<th>24 hrs</th>
<th>48 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0. untreated EFV</td>
<td></td>
<td>5.685 ± 0.0009</td>
<td>6.798 ± 0.0018</td>
</tr>
<tr>
<td>1. Form 1</td>
<td></td>
<td>5.492 ± 0.0016</td>
<td>6.597 ± 0.0017</td>
</tr>
<tr>
<td>2. Form 2</td>
<td></td>
<td>7.375 ± 0.0002</td>
<td>8.049 ± 0.0009</td>
</tr>
<tr>
<td>3. Form 3</td>
<td></td>
<td>6.467 ± 0.0034</td>
<td>6.804 ± 0.0044</td>
</tr>
<tr>
<td>4. Form 4</td>
<td></td>
<td>5.732 ± 0.0027</td>
<td>6.222 ± 0.0014</td>
</tr>
<tr>
<td>5. Form 5</td>
<td></td>
<td>6.399 ± 0.0006</td>
<td>7.263 ± 0.0014</td>
</tr>
<tr>
<td>6. Form 6</td>
<td></td>
<td>7.912 ± 0.0001</td>
<td>8.317 ± 0.0032</td>
</tr>
</tbody>
</table>

Table 2: Dissolution of the highest top 3 soluble of EFV polymorph

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>7±0.08%</td>
<td>7±0.04%</td>
<td>13±0.04%</td>
<td>20±0.48%</td>
</tr>
<tr>
<td>10</td>
<td>9±0.05%</td>
<td>12±0.05%</td>
<td>19±0.02%</td>
<td>26±0.24%</td>
</tr>
<tr>
<td>15</td>
<td>15±0.07%</td>
<td>16±0.03%</td>
<td>24±0.09%</td>
<td>28±0.14%</td>
</tr>
<tr>
<td>30</td>
<td>26±0.06%</td>
<td>21±0.09%</td>
<td>37±0.1%</td>
<td>34±0.16%</td>
</tr>
<tr>
<td>45</td>
<td>35±0.05%</td>
<td>33±0.001%</td>
<td>46±0.15%</td>
<td>38±0.2%</td>
</tr>
<tr>
<td>60</td>
<td>42±0.03%</td>
<td>45±0.2%</td>
<td>50±0.06%</td>
<td>43±0.39%</td>
</tr>
</tbody>
</table>

Fig. 4: Diffractogram PXRD of untreated EFV (0), Form 1 (1), Form 2 (2), Form 3 (3), Form 4 (4), Form 5 (5), Form 6 (6).

Fig. 5: Dissolution profile of Form 1, Form 2, Form 3 and Form 6.

DISCUSSION

As seen in fig. 1 those habits came from recrystallization methods give different appearances. The untreated EFV was looking uncertain forms but looks more clearly after recrystallization. Form 1 looks birefringent crystalline areas with interference colors interspersed with grain boundaries, but others show more clear as crystalline forms especially Form 2, 3, 4 and 5. Form 6 looks as a longitudinal section with cloudy interferences.
Generally from the spectrum ranges, all of the polymorphic forms has the similar spectral pattern indicated the compound not get reacted with a solvent to arise new spectrum. But if studied further shows there in wavenumbers at 480, 660, 1400, 1600, 1750, 2300, and 3300 cm\(^{-1}\) had differences appearance between the crystal studied. It seems to agree with the publish reported before. According to Mishra, 2012 the trifluoromethyl group which is directly connected to the oxazine ring has symmetric and asymmetric stretches, bends, rock and torsional modes are associated with it. The band around 480cm\(^{-1}\) represents of the rocking modes of CF3 to have variable magnitudes in CF3 containing benzene. The symmetric stretching mode \(\nu_{s}(CF3)\) appears in the range 660-800cm\(^{-1}\) and the asymmetric stretching \(\nu_{as}(CF3)\) arise in the range 1100-1200 cm\(^{-1}\). The corresponding values around 1400 cm\(^{-1}\) signify to benzoazine ring, oxazine ring and cyclopropyl ring groups vibrations which are in a different meaning. In benzoazine ring means for asymmetric stretch and deformation of the ring \(R_{1}[\nu_{(\text{ring})} + R_{1}[\nu_{(\text{CH})}]\). Whereas in oxazine ring means for ring deformation \(R_{2}[\nu_{(\text{NH})}]\). The while in cyclopropyl ring means for plane CH bending. The band values around 1600 cm\(^{-1}\) defined as a symmetric stretch of benzoazine ring \(R_{1}[\nu_{(\text{CC})}]\). The band around 1750 cm\(^{-1}\) means for oxazine ring stretch \(R_{2}[\nu_{(\text{CO})}]\). The stretching mode of the alkyl chain \((C=C)\) presented by a band around 2300 cm\(^{-1}\). At last the band around 3300 cm\(^{-1}\) depict of oxazine ring stretch vibration \(R_{2}[\nu_{(\text{NH})}]\).

For reason of the energy behaviour then DSC method was applied to samples. As shown in fig. 3 the thermogram of Form 2, 3 and 5 has more than an endothermic peak. It means those polymorphs were a metastable polymorph which can be transformed below the stable endothermic peak (138.6°C). From the images known that Form 2 and 3 almost has similar beginning transformed temperature (108.2°C and 108.4°C, respectively), but Form 2 followed by another transformed which almost same with Form 5 and its polymorph showed to correspond with the thermo-gram of DSC. From the table found the most solubility was Form 6, followed by Form 2, 3, 5 and 4, respectively. The previous publication was reported that EFV as Biopharmaceutical Classification System (BCS) class 2 has low aqueous solubility around 3-9µg/mL (Sathiga-ri, 2012). Found those polymorphs was appropriate with the range of solubility reported. For studied the stability among polymorphs in solution, the test continued till 48 hours were carried out. There are found all polymorphs stable enough in 48 hours time limit.

Found there are any differences, from the graph fig. 5, showed Form 3 was leading after 60 minutes test going on followed by Form 2 and 6. It looks the Form 6 at 25 minutes time early showed to more dissolve but then getting slowed and exceeded by Form 3 and 2, respectively.

**CONCLUSION**

The recrystallization methods with various solvents used have a modification of crystalline of EFV successfully. There are confirmed by different physicochemical analysis methods that the solvents used with experimental conditions affect to result in differences behaviors of the drug. Therefore, the solubility and dissolution rates among EFV polymorphs were slightly different and will affect to dosage form bioavailability. It concluded to inspection the EFV raw materials carefully.

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