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Eutectic Mixture of Fenofibric Acid and Syringic Acid: Improvement of Dissolution Rate and Its Antihyperlipidemic Activity

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Fenofibric acid is a poorly water-soluble drug with high permeability; thus, it is classified as a Biopharmaceutical Classification System (BCS) class II. This study aimed to prepare a eutectic mixture of fenofibric acid with syringic acid as a coformer to improve its solubility, dissolution rate, and antihyperlipidemic activity. The solvent co-evaporation method was used to form a eutectic mixture. Solid-state properties were characterized, solubility and in vitro dissolution profiles were studied, and in vivo antihyperlipidemic effectiveness was investigated in male Wistar rats. The results showed that the eutectic mixture of fenofibric acid-syringic acid enhanced the solubility (3.4-fold) and in vitro dissolution rate (3.62-fold) and significantly improved the antihyperlipidemic activity compared with intact fenofibric acid.

Introduction

Fenofibric acid (Figure 1A) is an active metabolite of fenofibrate that undergoes hydrolysis on its ester bond.[1] It possesses antihyperlipidemic activity by reducing total cholesterol levels, triglycerides, and apolipoprotein B.[2–4] However, according to the Biopharmaceutical Classification System (BCS), fenofibric acid is classified as a BCS class II drug due to its low aqueous solubility.[5] With a solubility of only 162.5 μg/mL in water and an absolute bioavailability of approximately 40% in animal studies, the dissolution rate of fenofibric acid is the rate-limiting step for its absorption in the gastrointestinal tract.[6,7] To address this limitation, efforts have been made to improve the solubility of fenofibric acid.

Various techniques have been employed to enhance the solubility of fenofibric acid, including the use of a $MgCO_3$ catalyst, ternary solid dispersion formation with hyaluronic acid and polyethylene glycol, and solid surface dispersion formation using croscarmellose sodium.[6,8,9] The preparation and characterization of multicomponent crystals, especially eutectic mixtures of fenofibric acid and syringic acid, have yet to be reported.

The formation of a multicomponent crystal can improve in vivo solubility and dissolution based on the crystalline intermolecular interactions.[10] A eutectic mixture, which is a combination of compounds that melt at the same temperature with a lower melting point than pure compounds, is a promising approach for improving drug solubility and dissolution. In this study, the eutectic mixture of fenofibric acid was produced using syringic acid as the coformer. A previous study showed that syringic acid can improve the solubility and dissolution profile of irbesartan.[11] In addition, syringic acid exhibits antioxidant and antihyperlipidemic activities, which may enhance the efficacy of fenofibric acid in the treatment of dyslipidemia.[12]

In this study, a eutectic mixture of fenofibric and syringic acids was prepared using the solvent co-evaporation method. The resulting mixture was characterized using Differential Scanning Calorimetry (DSC), X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), and Scanning Electron Microscopy (SEM), followed by an in vitro dissolution test and in vivo antihyperlipidemic activity evaluation in rats. To the best of our knowledge, the use of syringic acid as a coformer in eutectic mixtures with fenofibric acid has not been previously reported.

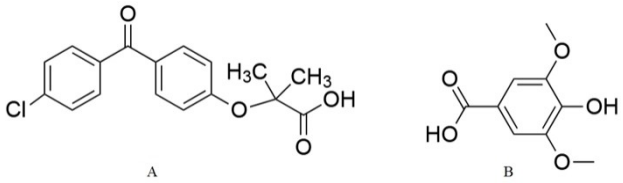

Figure 1. Molecular structure of A) fenofibric acid and B) syringic acid.

Results and Discussion

The binary phase diagram (Figure 2) was generated by plotting different molar ratios of fenofibric acid and syringic acid on the X-axis, and the endothermic peaks of the binary mixture on the Y-axis. The resulting binary phase diagram provides insight into the specific pattern of solid-state interactions between the active pharmaceutical ingredients and excipients.

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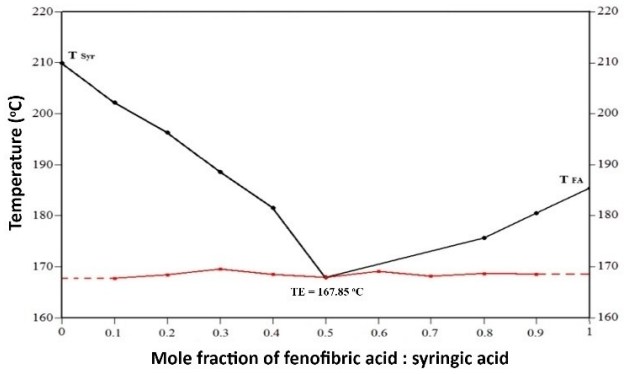

Figure 2. Binary phase diagram of fenofibric acid – syringic acid.

DSC thermograms of various molar ratios of the binary mixture of fenofibric acid and syringic acid are shown in Figure 3. Fenofibric acid has a melting point of 185.36 °C, while syringic acid exhibits a sharp endothermic peak at 209.62 °C due to a melting event. The molar ratio containing 0.9 to 0.6 of fenofibric acid in a binary mixture demonstrates two endothermic peaks. A new endothermic peak around 168.62–180.50 °C is attributed to the eutectic melting point. As the molar ratio of fenofibric acid decreased, the intensity of the second endothermic peak decreased. However, at an equimolar ratio of fenofibric acid and syringic acid, the thermal behavior of the binary mixture is significantly different from that of other compositions, appearing as a single endothermic peak at 167.85 °C, which is the eutectic point of the mixture. Moreover, if the molar ratio of fenofibric acid in the binary mixture (0.4 to 0.1) was lowered, the new endothermic peaks around 167.69–202.18 °C remained unchanged. However, the second endothermic peak shifted to a relatively higher temperature because of excess syringic acid in the binary mixture. In general, the binary phase diagram formed a V-shaped curve, proving that solid-state interactions occurred in fenofibric acid and syringic acid was a simple eutectic mixture. The co-crystallization process of two solid materials (drug-drug or drug-excipients) can generate diverse multicomponent crystals, including cocrystal/salt, eutectics, and solid solutions.[13,14]

A pure eutectic mixture of fenofibric acid and syringic acid (equimolar) was obtained by a solvent co-evaporation technique using ethanol as the solvent. In addition to its compatibility with a wide range of pharmaceuticals, ethanol is a polar solvent with a remarkable ability to dissolve many different compounds, including fenofibric acid and syringic acid. In addition, its low boiling point and relatively high evaporation rate make it easy to remove from the final product without damaging the API or the coformer. Additionally, ethanol is considered safe for use in humans, readily available, and relatively inexpensive.[15,16] After the eutectic mixture was obtained, the sample with the respective molar ratio was then characterized and used for further studies.

PXRD is an important tool for detecting changes in the solid crystalline phase during interactions between active pharmaceutical ingredients and excipients. New crystalline phases, such as cocrystals and salts, generate PXRD patterns that differ from those of their intact components. Meanwhile, eutectic mixtures and solid solutions do not change the PXRD patterns of the mixture.[17,18] Figure 4 shows the PXRD patterns of fenofibric acid, syringic acid, and the eutectic mixture. The PXRD patterns of fenofibric acid and syringic acid are similar to those reported in a recent study by Anggraini et. al.[19] and Haneef & Chadha.[20] The diffractogram of fenofibric acid shows intense and characteristic peaks at 2 theta = 15.8707, 18.4275, and 23.0899, while syringic acid demonstrates specific peaks at 2 theta = 13.4971 and 22.4931, indicating a highly crystalline nature. The eutectic mixture of fenofibric acid and syringic acid shows a superimposition of the characteristic peaks of the intact materials. However, in general, the intensities of the diffraction peaks decreased. These results prove that the co-crystallization of fenofibric acid and syringic acid produced a eutectic mixture rather than a new cocrystal phase.[21] The PXRD data support the thermal behavior analysis of the binary mixture mentioned earlier.

FTIR analysis was used to investigate the potential chemical interactions between fenofibric acid and syringic acid in the eutectic mixture. FTIR is a commonly used tool to study solid-state molecular interactions by analyzing the shift in the

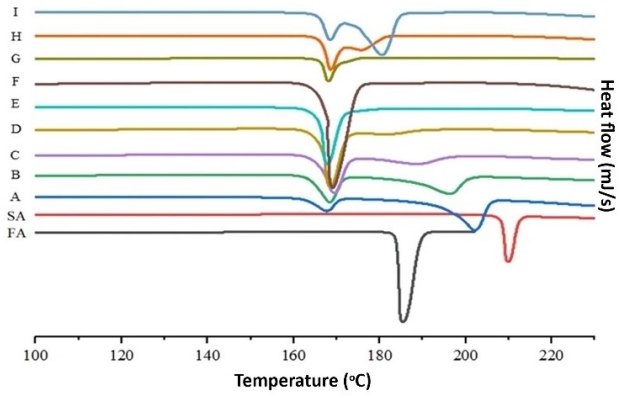

Figure 3. DSC thermograms of fenofibric acid, syringic acid, and molar fraction ratios of fenofibric acid and syringic acid of A) 0.1 : 0.9, B) 0.2 : 0.8, C) 0.3 : 0.7, D) 0.4 : 0.6, E) 0.5 : 0.5, F) 0.6 : 0.4, G) 0.7 : 0.3, H) 0.8 : 0.2, and I) 0.9 : 0.1.

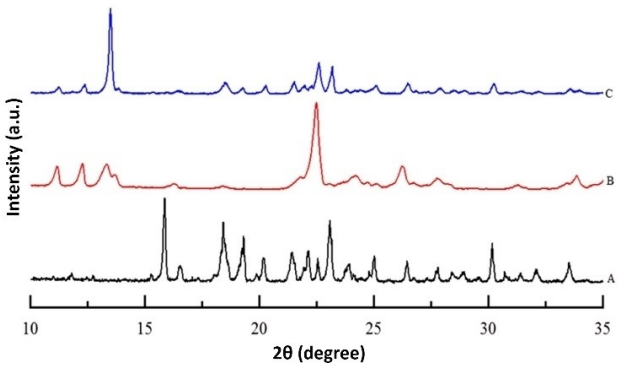

Figure 4. PXRD patterns of (A) fenofibric acid, (B) syringic acid, and (C) the eutectic mixture.

transmission bands in the FTIR spectrum. Figure 5 shows the wavenumber data for the eutectic mixture compared with the individual components of fenofibric acid and syringic acid. It has been reported that a slight shift in wavenumber indicates a weak hydrogen bond.[10] The FTIR spectra revealed a subtle shift in the wavenumbers of the eutectic mixture of fenofibric acid and syringic acid. However, they remained within the range of a functional group, indicating that there is no evidence of specific chemical interactions between fenofibric acid and syringic acid.

To better understand the shape and structure of the particles, we used scanning electron microscopy (SEM) to analyze fenofibric acid, syringic acid, and the eutectic mixture of fenofibric acid and syringic acid. The SEM images are presented in Figure 6. As shown in Figure 6A, fenofibric acid had an irregular polyhedral shape. In contrast, syringic acid (Figure 6B) was present as plate-shaped particles. Interestingly, the eutectic mixture of fenofibric acid and syringic acid (Figure 6C) exhibited a distinct needle-like shape, which was different from that of the intact particles of fenofibric acid and syringic acid. These findings suggest that the co-crystallization process of fenofibric acid and syringic acid produces a unique particle shape that differs from that of individual particles.

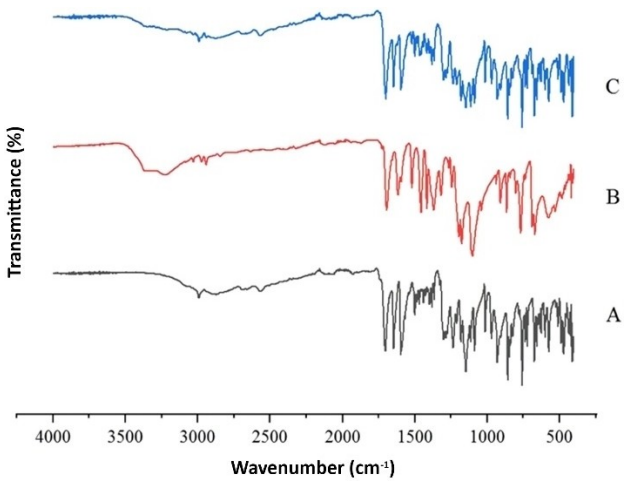

Figure 5. FT-IR spectra of (A) fenofibric acid, (B) syringic acid, and (C) the eutectic mixture of fenofibric acid and syringic acid.

Table 1. Solubility of fenofibric acid and its physical and eutectic mixtures with syringic acid.

Compound	Solubility (mg/100 mL)	Increase in solubility
Fenofibric Acid	1.580 ± 0.22^{a}	–
Physical mixture	2.120 ± 0.02^{b}	1.4-fold
Fenofibric acid – syringic acid eutectic mixture	5.403 ± 0.13^{c}	3.4-fold

Solubility data are expressed as mean ± SD; the increase in solubility is compared with the solubility of fenofibric acid; different superscripts indicate a significant difference (analyzed with one-way ANOVA followed by Duncan's MRT with 95% confidence interval, n = 9).

The solubility and dissolution rates of the different samples were investigated, and the results are summarized in Table 1 and Figure 7. The solubility of fenofibric acid was significantly improved in both the physical mixture and eutectic mixture with syringic acid, and the eutectic mixture showed a remarkable 3.4-fold increase compared with intact fenofibric acid. Similarly, the dissolution rate of fenofibric acid was significantly higher in the eutectic mixture than in its intact form, with almost 93.87% dissolution of the eutectic mixture within 30 min, whereas only 25.92% of the intact fenofibric acid dissolved during the same period.

The WHO biowaiver dissolution criterion states that an immediate release (IR) solid oral dosage form should exhibit 'very rapid' (no less than 85% of the drug must be dissolved in 15 min) or 'rapid' (no less than 85% of the drug must be dissolved in 30 min) in vitro dissolution kinetics.[22] The significant increase in the dissolution rate of fenofibric acid from the eutectic mixture with syringic acid was due to the local solubilization effect produced by syringic acid in the diffusion layer surrounding the fenofibric acid particles. This effect enhanced the solubility of fenofibric acid in the dissolution medium, resulting in a faster dissolution rate. Furthermore, the increase in dissolution rate could be attributed to the alteration of thermodynamic properties of the eutectic mixture, such as

Figure 6. Scanning electron microphotographs of (A) fenofibric acid, (B) syringic acid, and (C) eutectic mixture of fenofibric acid – syringic acid (500× magnification).

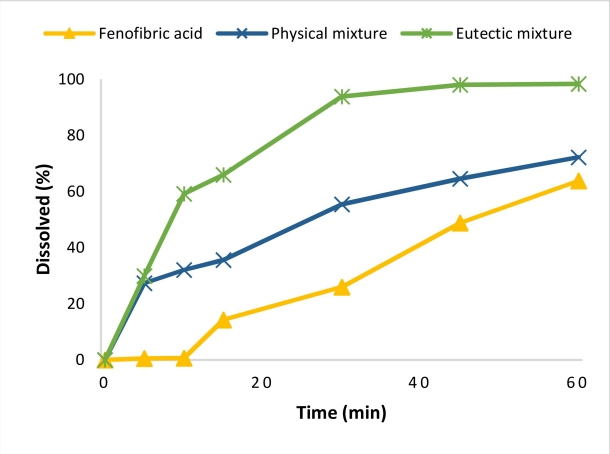

Figure 7. Dissolution rate profile of fenofibric acid, its physical and eutectic mixtures with syringic acid, obtained using HPLC. The molar ratios of fenofibric acid and syringic acid for both physical and eutectic mixtures were 0.5 : 0.5, respectively (n = 18).

high free energy, greater molecular mobility, and weaker intermolecular interaction[20,23]

To determine the correlation between the solubility and dissolution rate of a eutectic mixture of fenofibric acid – syringic acid and antihyperlipidemic effectiveness, we conducted a study of antihyperlipidemic activity in rats. The total cholesterol levels of the group receiving the eutectic mixture of fenofibric acid – syringic acid were significantly reduced compared with those of the group receiving fenofibric acid. The results showed that the reduction in total cholesterol levels after 15 days in the fenofibric acid and eutectic mixture groups were 41.79% and 56.03%, respectively (Figure 8). These results suggest that solubility and dissolution rate significantly affect the in vivo antihyperlipidemic activity of fenofibric acid.

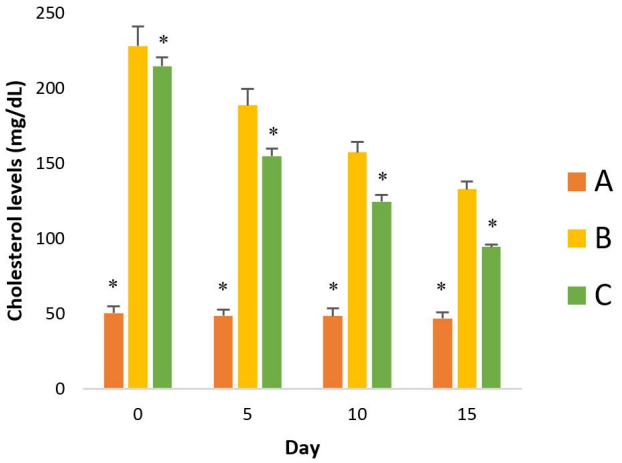

Figure 8. Changes in cholesterol levels in rats over time in response to different treatments for 15 days: (A) control, (B) fenofibric acid, and (C) eutectic mixture of fenofibric acid and syringic acid. (*) denotes a statistically significant difference ($p < 0.05$) compared with fenofibric acid (n = 72).

Increased solubility and dissolution rates are required to achieve optimal bioavailability and effectiveness of poorly soluble drugs.[24]

Conclusion

The eutectic mixture of fenofibric acid and syringic acid is a multicomponent crystal that significantly enhances the solubility and dissolution rate of fenofibric acid. When tested on hyperlipidemic rats induced with an atherogenic cocktail, the eutectic mixture was found to be significantly more effective than intact fenofibric acid in reducing the total cholesterol levels. This finding highlights the potential of eutectic mixtures in the development of more effective antihyperlipidemic drugs.

Experimental Section

Materials

Fenofibric acid was purchased from BOC Sciences (New York, USA). Syringic acid was obtained from the Tokyo Chemical Industry (Tokyo, Japan). The cholesterol reagent CHOD-PAP was a product of Greiner Laboratories GmbH (Germany). Ethanol, acetonitrile, and ethanol of HPLC grade were purchased from Merck (Darmstadt, Germany). All solvents used were of analytical grade.

Methods

Differential Scanning Calorimetry (DSC) analysis

Thermal analysis was performed using differential scanning calorimetry (Shimadzu DSC-06 Plus, Japan). The eutectic mixture samples were sealed in an aluminum pan, and the DSC apparatus was heated in the temperature range of 30 °C to 250 °C at a heating rate of 10 °C/min.

The binary mixtures of fenofibric acid and syringic acid were prepared at various molar ratios (ranging from 0.1 : 0.9 to 0.9 : 0.1). The endothermic peaks of each mixture were determined using a DSC apparatus. A phase diagram was constructed by plotting the endothermic peak of the eutectic mixture against the molar ratio.

Powder X-ray Diffraction (PXRD) analysis

Powder X-ray diffraction analysis was carried out at room temperature using a PANalytical PW 30/40 X-ray diffractometer (Netherlands) with metal Cu, Kα filter, voltage 45 kV, current 40 mA, and in the range of 2 theta 5°–45°.

Fourier transform infrared (FT-IR) spectroscopy analysis

The sample was measured using an infrared spectrophotometer (Shimadzu IR Tracer-100 AH, Japan) by dispersing it on a KBr plate that was compressed at high pressure (hydraulic press). The absorption spectra were recorded at wavenumbers of 4000–400 cm^{-1}.

Scanning Electron Microscopy (SEM)

The morphology of the eutectic mixtures was characterized using a Scanning Electron Microscope (HITACHI FLEXSEM 1000, Japan) at

an accelerating voltage of 10 kV. The samples were placed in the sample holder and sprayed with a thin gold-palladium film. The measurement conditions were set to 10 kV and 12 mA.

Solubility studies

An excess amount of fenofibric acid, the eutectic mixture, and the physical mixture of fenofibric acid and syringic acid were added to 100 mL of distilled water in an Erlenmeyer flask. The samples were shaken using a sonicator at ambient temperature for 5 minutes and filtered through a membrane (0.45 μm PTFE filter). The concentration of fenofibric acid was determined using an HPLC system (Shimadzu, Japan) equipped with a DAD UV-Vis detector. The HPLC system used XRS C_{18} 4.6×150 pursuit columns. The mobile phase was a mixture of acetonitrile and distilled water adjusted to pH 3 (70:30, v/v) at a flow rate of 1 mL/min, and the eluent was detected at 287 nm for the analysis of fenofibric acid. The retention time of fenofibric acid was 6.353 min. All experiments were carried out in triplicates.

In vitro dissolution rate studies

Fenofibric acid, the eutectic mixture, and the physical mixture of fenofibric acid and syringic acid (equivalent to 105 mg of drug) were each filled in hard gelatin capsules. The dissolution test was performed using a USP type 1 dissolution apparatus (SR8-Plus Dissolution Test Station Hanson Research, USA) at a speed of 50 rpm at 37±0.5 °C in 900 mL of phosphate buffer medium (pH 6.8) for 60 min. Samples (5 mL) were collected at 5, 10, 15, 30, 45, and 60 min. The samples were filtered through a 0.45 μm PTFE membrane filter. The fenofibric acid concentration in the filtrate was analyzed using the validated HPLC method described above.

In vivo antihyperlipidemic activity

The *in vivo* antihyperlipidemic effectiveness of the multicomponent crystal was investigated in male Wistar rats aged 3–4 months and weighing 250–300 g. The animals were acclimatized for 7 days and then induced with an atherogenic cocktail 1% of their body weight consisting of 100 g cholesterol and 30 g propylthiouracil in 1 L peanut oil for 7 days. The animals were divided into three groups that received a daily dose of vehicle control (NaCMC suspension 1%), fenofibric acid, and the eutectic mixture (doses were equivalent to fenofibric acid 9.45 mg/kg body weight) for 15 days. Blood was withdrawn by the retro-orbital technique using capillary tubes on 6th, 11th, and 16th day, then centrifuged at 3000 rpm for 15 min. The serum was transferred to a microtube and added with the reagent to measure the blood cholesterol levels using a photometer 5010V5+ apparatus (Robert Riele KG, Berlin). The experimental protocol was approved by the Ethics Committee of the Faculty of Medicine, Universitas Andalas No. 61/UN.16.2/Kep-FK/2020.

Statistical analysis

The solubility study data were analyzed using one-way ANOVA and expressed as mean±standard deviation (SD). The dissolution rate data were plotted as time-curve charts. Blood cholesterol levels of the rats were analyzed using two-way ANOVA and are presented in a bar chart. Both ANOVA analyses were followed by Duncan's multiple range test (MRT) to determine significant differences between groups. All data analyses were performed using the IBM SPSS Statistics version 28 (IBM, USA). The significance level was set at $P < 0.05$.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: dissolution rate · eutectic mixture · fenofibric acid · in vivo antihyperlipidemic · syringic acid

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