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FORMULATION AND EVALUATION OF ETOFIBRATE SELF-EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS)

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ABSTRACT

Aim: The aim of the current study is to develop self-emulsifying drug delivery systems (SEDDS) of etofibrate to enhance the dissolution of poorly soluble etofibrate. **Methods:** The pseudo-ternary phase diagram was constructed to find the optimised formulation. The characterization of the selected formulations were evaluated including the droplet size after dispersion, zeta potential, drug content and *in vitro* dissolution. **Results:** The SEDDS were developed using oleic acid, Tween-80 and isopropanol as oil, surfactant and co-surfactant respectively. The dissolution test demonstrated that nearly 90% of etofibrate were dissolved from SEDDS within 60 min while only about 15% of raw drug were dissolved in 60 min. Further, the stability studies for 6 months revealed that SEDDS of etofibrate are found to be stable without any significant change in physicochemical properties. **Conclusion:** The prepared SEDDS enhance the dissolution of poorly soluble drug and has a potential to enhance drug absorption and improve bioavailability of drug.3

KEY WORDS: Etofibrate, SEDDS, Pseudo-ternary phase diagram, Dissolution.

INTRODUCTION

Etofibrate (Fig. 1), 2-(p-chlorophenoxy)-2-methylpropionic acid 2-(nicotinoyl-oxy)ethyl ester, a derivative of clofibrate and nicotinic acid, is generally used for adjusting blood fat.[1] The drug is decomposed into clofibrate and nicotinic acid by enzymes in the body and then quickly produces lasting lipid-lowering effect after it is taken. Its main pharmacological effects include inhibiting synthesis of cholesterol, triglyceride and promoting steroid excretion. Clinically, etofibrate mainly lower triglyceride of human plasma for the treatment of hyperlipidemia. However, as poor water-soluble drug, low dissolubility limits its clinical application. With the objective of improving the poor solubility and dissolution, properties of drug, advanced drug delivery systems are requisite.

SEDDS are the isotropic mixtures of drug, lipid and surfactants, usually with one or more hydrophilic co-solvents or co-emulsifiers which upon mild agitation generates ultrafine droplets of oil in water emulsion.[2] This type of drug delivery system has significant advantages as follows. On one hand, SEDDS can avoid the inactivation and enzymatic degradation of peptide and protein drugs in gastrointestinal tract. Meanwhile, the absorption and pharmacological activity of the drugs can be significantly increased compare with other formulations.[3-5] On the one hand, the free energy required for self-emulsification process is low.[6] SEDDS can realize the spontaneous emulsification due to the gastrointestinal motility *in vivo* instead of artificial emulsification *in vitro*.[7] The enhanced interfacial area of micronized globules will facilitate the dissolution of drug thereby improving the bioavailability and enhance permeability through biological membranes due to presence of lipid and surfactant.[8] SEDDS is more stable compared with conventional thermodynamically unstable multiple emulsions[9] and it can effectively avoid the poor stability of multiple emulsions during preparation and storage *in vitro*. Besides, SEDDS greatly facilitates the patients by reducing the dose volume.[10]

Therefore, the present investigation was carried out to improve dissolution characteristics of etofibrate by preparing SEDDS. It may the foundation for further study of the formulation in the long run.

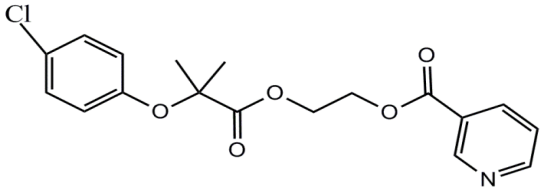


Fig.1 Structure of etofibrate.

MATERIALS AND METHODS

The reference substances of etofibrate were purchased from National Institute for Control of Pharmaceutical

and Biological Products (Beijing, China). Oleic acid, ethyl oleate, IPM, Tween-80, OP-10, EL, absolute ethyl alcohol, isopropanol, glycerinum were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solvents were of analytical reagent grade and were used as received. Double distilled water is also used.

METHODOLOGY

HPLC determination of etofibrate

The detect of wave length

Weigh etofibrate reference substance precisely and dissolve with ethanol and compared to blank ethanol solution, the wavelength was scanned by ultraviolet spectrophotometer in range from 200nm to 800nm. Result showed etofibrate reference substance had maximum absorption peak in 222nm. Therefore 222nm is the suitable detect length for detection of etofibrate.

Chromatographic condition

An HPLC system equipped with YMC-Pack NH2 column (250mm×4.6mm i.d.) was used for the determination of etofibrate. The mobile phase consisted of acetonitrile:absolute ethyl alcohol:0.1%phosphoric acid aqueous solution at a volumetric ratio of 80:10:10. The elution was carried out at a flow rate of 1.0 mL/min at room temperature and the detect wavelength was 222nm.

Etofibrate reference substance solution

Weigh 20.0mg etofibrate reference substance precisely and put it in 100ml volumetric flask and then dissolve with amount of the mobile phase and dilute to scale, therefore get 0.2 mg/mL etofibrate reference substance solution.

Etofibrate self-emulsifying sample

Carry out etofibrate self-emulsifying preparation according to the best prescriptions and dilute to scale therefore getting etofibrate self-emulsifying sample.

Negative standard solution free from etofibrate

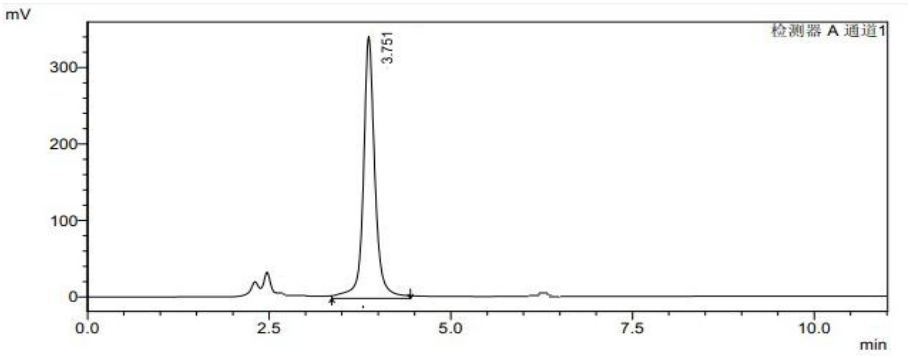
Weigh various accessories, dissolve with the mobile phase, therefore getting negative standard solution free from etofibrate.

The calibration curves

The calibration curves were drawn to measure the concentration of the samples. Etofibrate reference substance solution at the volume of 1, 2, 4, 6, and 8 mL was transferred to a 10 mL volumetric flask and added additional mobile phase to the mark and shaking, respectively. Then a 50 µL solution was injected into the HPLC system for analysis. The peak area correlated linearly with etofibrate concentration in the range of 0.02-0.2 mg/mL and the average correlation coefficient was 0.9999.[11]

Specificity of etofibrate self-emulsifying preparation

Etofibrate reference substance solution, etofibrate self-emulsifying sample and negative standard solution free from etofibrate were respectively injected into the HPLC system for analysis according to the chromatographic conditions. Results showed that negative standard solution free from etofibrate had no absorption peak in the maximum absorption peak. Etofibrate self-emulsifying sample had strong absorption and good relative peak separation in the corresponding retention time. Therefore accessories had freedom from interference (Figure 2).



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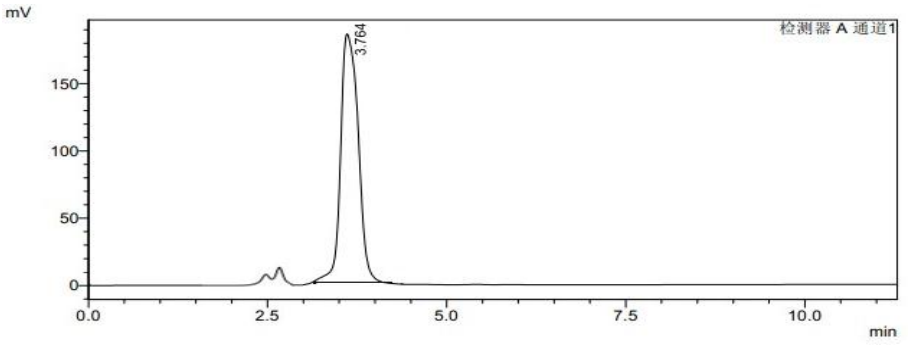

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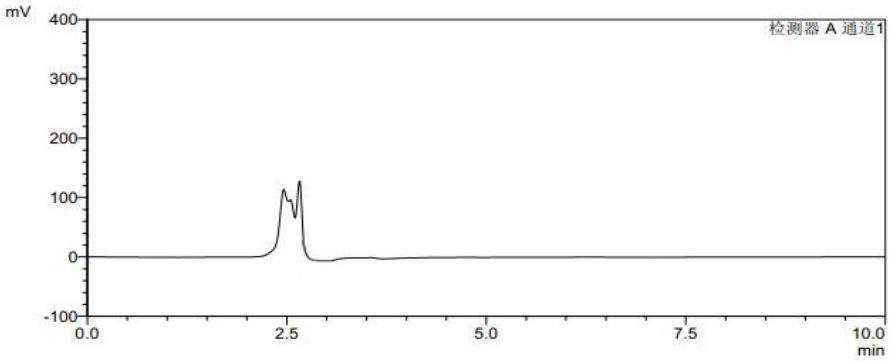

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Figure 2: Specificity of etofibrate self-emulsifying preparation.

A. Etofibrate reference substance solution **B**. Etofibrate self-emulsifying sample **C**. Negative standard solution free from etofibrate.

METHODS

Solubility studies

Weigh different recipients 1.0g in the test tube, excessive etofibrate were added. The mixture was vortexed using a mixer at a maximum speed for 10 min and kept for 48 h at 37 °C in a shaking water bath to facilitate the solubilization. The samples were centrifuged at 3000rpm for 15 min. The supernatant was taken and diluted with the mobile phase for solubility quantification of etofibrate in different accessories by HPLC.

Construction of pseudo-ternary phase diagrams

The pseudo-ternary phase diagrams of oil, surfactant/co-surfactant and water were developed using the water titration method. Mixtures of oil and surfactant/co-surfactant at certain weight ratios were weighed precisely and mixed at mass ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. Then keep surfactant and co-surfactant constant and make experiments according to above methods. Each mixture was observed visually. The tendency to emulsify was judged good when droplets spread easily in water and formed a fine bluish emulsion [12]. For each phase, diagrams at a specific ratio of surfactant/co-surfactant, 1:1, (w/w) were used.

The pseudo-ternary phase diagrams were established for each oil, surfactant and co-surfactant and the final oil, surfactant and co-surfactant were selected based on the area of emulsion. The emulsion formulations were selected at desired component ratios.

Formulation optimization

Mixtures of optimized oil and surfactant/co-surfactant at certain weight ratios were weighed precisely and mixed at mass ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. The mixture was stirred at 37 °C for 10 min in a shaking water bath and added 50ml double distilled water. The appearance, the emulsifying time, the values of z-average diameters were recorded thus getting the formulation optimization.

The determination of prescription

The oil and the mixed emulsifiers were weighed at mass ratios of 3:7 and the mixture were added etofibrate at certain ratio. The appearance, the emulsifying time, the values of z-average diameters were observed.

Preparation of etofibrate-SEDDS

SEDDS formulations with etofibrate were prepared by dissolving the drug into the mixture of surfactant, oil and co-surfactant with heating in a water bath of 37 °C and vigorous vortexing until all of the drug material was completely dissolved. The resultant mixture was vortexed until a clear bluish solution was obtained and stored at room temperature until further use (Figure 3).

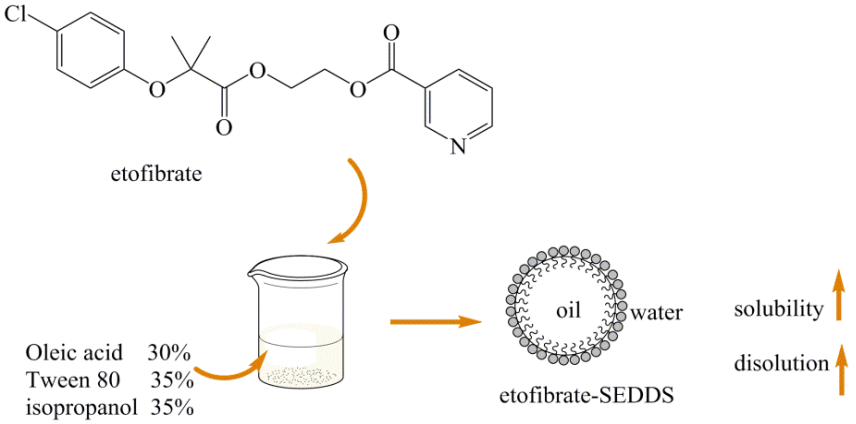

Figure 3: Preparation of etofibrate-SEDDS.

Evaluation of etofibrate self-emulsifying drugs

Identification of types

The types of etofibrate self-emulsifying drugs were identified by dilution method and staining method.

Dilution method: A large amount of water was added to the etofibrate self-emulsifying drugs. If they could be diluted infinitely, they were o/w emulsion.

Staining method: Sudan (red) and methylene blue (blue) were added to the etofibrate self-emulsifying drugs. If the diffusion rate of blue is greater than red, they were o/w emulsion.

Droplet size and zeta potential determination

The droplet size distribution and zeta potential values of the emulsions resulting from the self-emulsification of the SEDDS in water were measured by dynamic light scattering. All measures were repeated three times, and the values of z-average diameters (nm) and zeta potential (mv) were recorded.

Drug content

The optimised SEDDS containing 2g etofibrate was dispersed into appropriate quantity of the mobile phase, stirred sufficiently to dissolve the drug and centrifuged at 3000 rpm for 15 min. The supernatant was duly diluted and analyzed at λmax of 220 nm by HPLC method. Three batches of samples (batch number: 20150911, 20150912, 20150913) were determined.

Stability studies

Stable SEDDS can be generally stored for a long time under normal storage conditions. Properties such as content and size after dilution should not be significantly changed. In the current research, the stability of SEDDS was evaluated for six months. Three batches of samples (batch number: 20150911, 20150912, 20150913) were collected after 0, 1, 2, 3 and 6 months. The appearance, self-emulsifying properties, emulsion size and drug content were evaluated.

***In vitro* dissolution test**

The dissolution profiles of raw etofibrate, liquid SEDDS, were respectively obtained using a USPII paddle method (100 rpm, 37 °C ± 0.5 °C, 900 mL dissolution medium, as following purified water, simulated gastric fluid, simulated intestinal fluid) with a ZRS-8G dissolution tester. Samples containing equivalent amounts of etofibrate (50 mg) were added to each vessel. Aliquot samples (5 mL) were withdrawn at predetermined time intervals (5, 10, 20, 30, 60 min) and filtered through a 0.22 μm filter membrane, assayed by HPLC at a wavelength of 222 nm. An equal volume of fresh medium was added to compensate the loss due to sampling. Each dissolution test was performed in triplicate.[13]

RESULTS AND DISCUSSION

Solubility studies

The self-emulsifying formulations consisting of oil, surfactants, co surfactant and drug should be a clear and monophasic liquid at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the drug in solution.[14] Comparative study of solubility of etofibrate in various vehicles is given in the **Figure 4**. Tween-80 was found to be the highest solubility in surfactants, absolute ethyl alcohol had higher solubility than isopropanol, but isopropanol increase patient compliance. So Tween-80 and isopropanol were chosed as surfactant and co-surfactant respectively. Oleic acid and ethyl oleate had similar solubility among the oils. They were further utilized for the construction of ternary phase diagram.

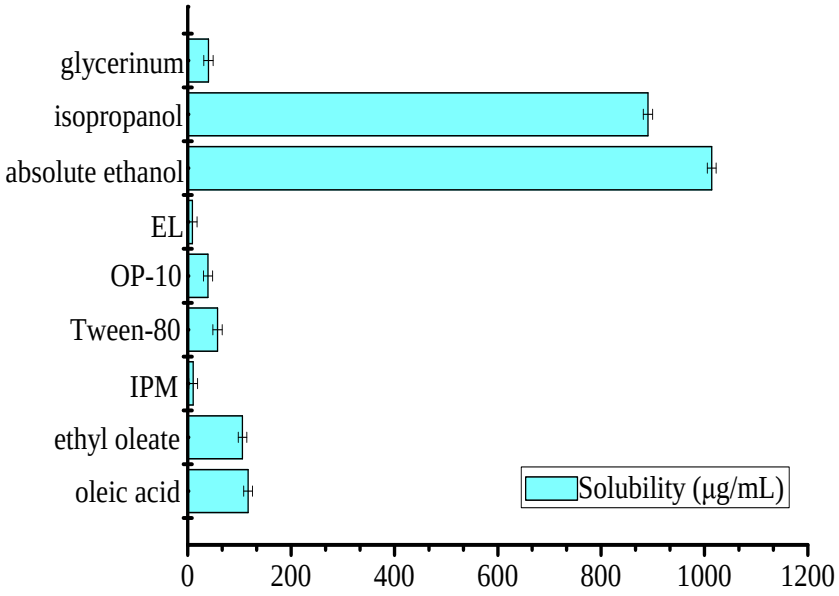

Figure 4: Solubility of etofibrate in various vehicles.

Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed to identify the emulsion regions and optimize the oil vehicles. The best ratios of the excipients obtained from these diagrams were used to obtain the emulsion regions area.[15] **Figure 5** shows the phase diagrams of systems with different oils. Oleic acid had larger emulsion regions than ethyl oleate, so oleic acid was chose as oil of the prescription.

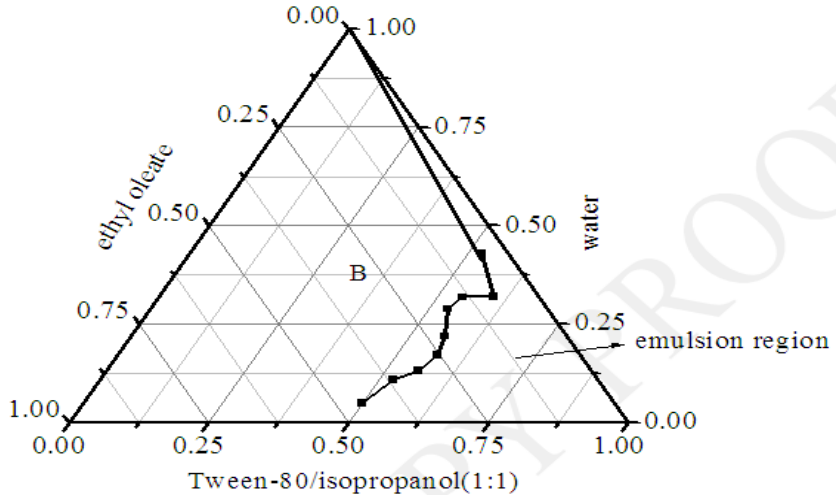

Figure 5: The pseudo-ternary phase diagrams of different oils.

Formulation optimization

As was shown in **Table 1**, the formulation had transparent and light blue opalescence appearance and small Z-average diameters when proportion was 3:7.

Table 1: Formulation optimization（n=3）

Proportion	Oleic acid/g	Tween80/g	Isopropanol/g	Emulsifying time/s	Emulsifying appearance	Z-average diameters /nm	RSD/%
1:9	0.398	1.802	1.801	65	Muddy, oyster white	135	0.28
2:8	0.809	1.603	1.598	31	Semitransparent, light blue opalescence	163	0.17
3:7	1.201	1.405	1.403	21	Transparent, light blue opalescence	119	0.12
4:6	1.607	1.203	1.201	25	Semitransparent, light blue opalescence	153	0.10
5:5	2.004	1.006	1.000	27	oyster white transparent	175	0.19
6:4	2.408	0.805	0.803	29	oyster white transparent	193	0.20
7:3	2.803	0.605	0.602	37	oyster white haze	149	0.27
8:2	3.201	0.402	0.407	35	oyster white haze	165	0.24
9:1	3.605	0.201	0.204	—	layered	—	—

The determination of the prescription

As shown from **Table 2**, when etofibrate was added 2g, the prescription could form SEDDS with light blue opalescence. Therefore, the best prescription was used as etofibrate:oleic acid: Tween- 80: Isopropanol(20:30:35: 35).

Table 2: The determination of the prescription（n=3）

Etofibrate/g	Oleic acid/g	Tween-80/g	Isopropanol/g	Emulsifying time/s	Emulsifying appearance	Particle size/nm	RSD/%
0.102	1.201	1.402	1.403	21	Transparent, light blue opalescence	103	0.05
0.501	1.204	1.405	1.407	23	Transparent, light blue opalescence	104	0.08
1.003	1.201	1.403	1.402	25	Transparent, light blue opalescence	115	0.14
1.504	1.200	1.402	1.401	27	Transparent, light blue opalescence	117	0.23
2.003	1.205	1.402	1.407	28	Transparent, light blue opalescence	123	0.26

2.501	1.200	1.401	1.403	45	Oyster white haze	293	0.28
3.002	1.203	1.404	1.402	67	Oyster white haze	349	0.35

Evaluation of formulation characteristics

Identification of types

Results showed the etofibrate self-emulsifying drugs could be diluted infinitely and the diffusion rate of blue is greater than red. Therefore, the prepared emulsions were o/w emulsions.

Droplet size and zeta potential determination

The rate and extend of drug release as well as absorption mainly depends upon the globule size of the emulsion. Hence, globule size determination is a crucial factor for self emulsifying drug delivery system.[16] The results of TEM and the mean diameters of the etofibrate self-emulsifying drugs are shown in **Figure 6**. The mean sizes were 123.2nm and the zeta potential values were -15.3 mV.

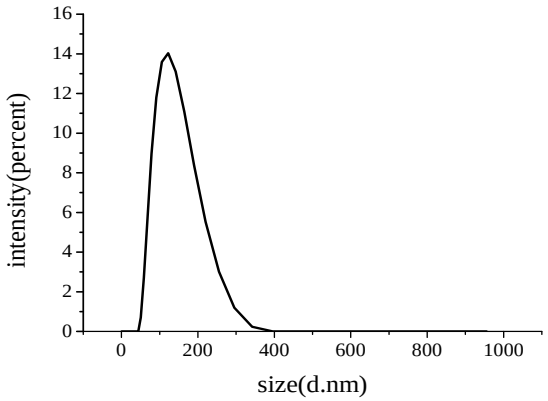

Figure 6: Particle size of etofibrate-SEDDS.

Drug content

The mass concentrations of etofibrate were figured up according to etofibrate standard calibration curve .The results were 188.3 μg/mL, 187.3μg/mL, 188.9μg/mL and the mean drug content was 188.1μg/mL.

Table 3: The drug content of etofibrate.

Lot number	20150911	20150912	20150913	Mean value	RSD(%)
Drug content(μg/mL)	188.3	187.3	188.9	188.1	0.37
Amount（%）	94.15	93.65	94.45	94.08	0.43

***In vitro* dissolution test**

All the liquid formulations showed good self emulsification efficiency forming nanoemulsion immediately after dilution. The *In vitro* drug release study data as shown in **Figure 7**. The dissolution enhancement with more than 90% drug release in initial 60 min, while pure drug release were found to be only 15% within 60min. Therefore, etofibrate SEDDS increase the dissolution of etofibrate.

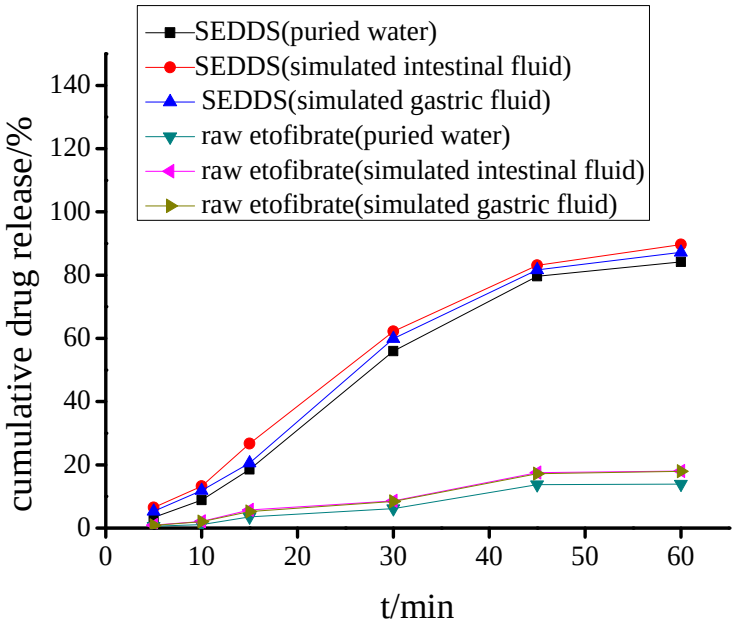

Figure 7: *In vitro* dissolution test.

Stability studies

The results are shown in **Table 5**. The etofibrate content in the SEDDS remained similar which reflected that the optimized formulation was stable under the experiment condition. Furthermore, no significant change was found in the appearance, drug content, and particle size.

Table 5: Stability studies.

Batch number	Time/ month	Appearance	Particle size/nm	Content (mg/g)
	0	Transparent, light blue opalescence	119	1.87
	1	Transparent, light blue opalescence	124	1.86
20150911	2	Transparent, light blue opalescence	120	1.87
	3	Transparent, light blue opalescence	122	1.85
	6	Transparent, light blue opalescence	123	1.82
	0	Transparent, light blue opalescence	125	1.86
	1	Transparent, light blue opalescence	118	1.83
20150912	2	Transparent, light blue opalescence	123	1.81
	3	Transparent, light blue opalescence	126	1.79
	6	Transparent, light blue opalescence	120	1.77
	0	Transparent, light blue opalescence	121	1.85
	1	Transparent, light blue opalescence	118	1.82
20150913	2	Transparent, light blue opalescence	120	1.78
	3	Transparent, light blue opalescence	126	1.76
	6	Transparent, light blue opalescence	128	1.75

CONCLUSION

SEDDS formulation of etofibrate composing of oil, surfactant and co-surfactant was used to improve the dissolution of etofibrate. The optimal concentration of components was determined using solubility study and the pseudo-ternary phase diagram. The formulation containing oleic acid, Tween-80 and isopropanol (30:35:35) demonstrated the highest drug dissolution in 60 min, resulting from a fast spontaneous emulsion formation and small droplet size. The results of this study suggest the potential use of developed SEDDS formulation for the delivery of poorly water-soluble drug etofibrate. Hence, it has a potential to enhance drug absorption and bioavailability.

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