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A high-throughput approach towards a novel formulation of fenofibrate in omega-3 oil

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ABSTRACT

A novel lipid formulation containing fenofibrate in omega-3 oil was developed using a novel high-throughput screening platform. The optimized formulation combines the cardiovascular health benefits from omega-3 oil with the potent lipid-regulating effect of fenofibrate. When tested against the current marketed product Tricor® in healthy human volunteers, the new formulation was shown to be equivalent to Tricor®.

1. Introduction

Fenofibrate is a well studied potent lipid-regulating agent for the treatment of hypercholesterolemia and hypertriglyceridemia (http://www.rxabbott.com/pdf/tricorpi.pdf) that is primarily marketed under the brand Tricor®. Clinical studies have shown that fenofibrate is effective in reducing total cholesterol, low-density lipoprotein (LDL) cholesterol, apolipoprotein B, total triglycerides and triglyceride-rich lipoprotein.

Fenofibrate is crystalline at room temperature and melts at 80 °C. The compound (Fig. 1), an iso-propyl ester pro-drug of the active component, fenofibric acid, is poorly water soluble (<1 μg/ml). Permeability across gastrointestinal tract (GI) is considered high as evidenced by successive decreases in Tricor® dose while maintaining the same exposure via reduction in drug particle sizes.

Despite its low water solubility, fenofibrate shows relatively high solubility in many lipid excipients. It is especially soluble in oils such as monoglycerides and ethyl esters of fatty acids.

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Abbreviations: ANOVA, analysis of variance; AUC, area under the plasma *vs.* time curve a measure of drug exposure; CHD, coronary heart disease; DHA, docosahexaenoic acid; EPA, eicosapentanoic acid; GI, gastrointestinal tract; HLB, hydrophilic–lipophilic balance; HPLC, high-performance liquid chromatography; HT, high-throughput; LDL, low-density lipoprotein; RRT, relative retention time; SEDDS, self-emulsifying drug-delivery system; SGF, simulated gastric fluid.

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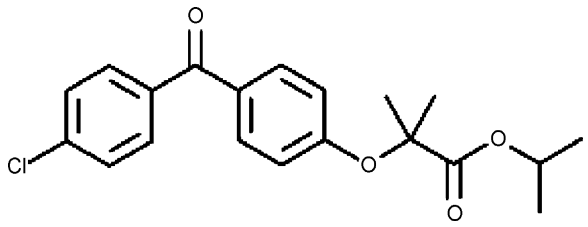


Fig. 1 – Fenofibrate chemical structure.

In the solubilized form, the need for dissolution is removed and fenofibrate can be expected to have a greater opportunity for absorption. This enables novel formulation strategies that can be alternatives to the particle size reduction techniques that have been used.

Omega-3 oils and omega-3 ethyl esters are lipid vehicles of particular interest to dissolve fenofibrate due to their potential cardiovascular health benefits (Ascherio et al., 1995; Daviglus et al., 1997; Harper and Jacobson, 2005; GISSI, 1999) stemming from their high polyunsaturated fatty acids (PUFA) content, namely docosahexaenoic acid (DHA) and eicosapentanoic acid (EPA). These benefits have been the focus of numerous clinical trials across various ethnic groups investigating the role of omega-3 oils in preventing coronary heart diseases (CHD). The largest and most extensive was the Gruppo Italiano per 10 Studio della Sopravvienza nell'Infarcto Miocardio (GISSI) trial which followed approximately 10,000 patients for 3.5 years (GISSI, 1999). The results from these trials demonstrated that regular uptake of omega-3 oils from fatty fish can significantly reduce total mortality, coronary heart disease death, and sudden death (GISSI, 1999).

This information led to the novel product concept of formulating fenofibrate in omega-3 oil. In addition to the lipid-regulating effect from fenofibrate, regular intake of the omega-3 oil as a supplement may yield added benefits as described above. Therefore, the goal for this study is to develop a self-emulsifying formulation of fenofibrate in omega-3 oil which delivers the current marketed dose of 145 mg fenofibrate.

Lipid formulations can be categorized into three different types as shown in Table 1 based on their composition and physical characteristics (Pouton, 2000).

Formulations that simply contain lipids without surfactants or cosolvents were categorized as Type I. Here, the formulation relies on natural emulsifiers along with gentle movements in the GI tract to help disperse the contents. Type II formulations contain surfactants to promote self-dispersion. This type is also known as self-emulsifying drug delivery systems (SEDDS). In addition to surfactants, Type III formulations also contain varying amounts of hydrophilic cosolvents which may help increase drug solubility or control formulation properties.

A self-emulsifying formulation was targeted for our formulation due to its ability to form fine colloidal droplets with very high surface area. In many cases, this accelerates the digestion of the lipid formulation, improves absorption, and reduces food effect and inter-subject variability (see for example Constantinides, 1995; Humberstone and Charman, 1997; Pouton, 1997, 2000; references therein).

For the design of our SEDDS formulation, it was also desirable to include as much omega-3 oil as possible to take advantage of its health benefits (Krauss et al., 2000). As a result, it was necessary to minimize the amount of non-lipid excipients included in the formulation while maintaining good dispersion characteristics.

This paper describes the design of a novel SEDDS formulation of fenofibrate using high-throughput (HT) experimentation. To find self-emulsifying formulations of fenofibrate with suitable dispersion characteristics, a proprietary HT formulation platform was utilized to create and evaluate 768 excipient combinations. The experimental design focused on studying the effects of each excipient on the formulation miscibility and dispersion characteristics of the excipient mixtures. The knowledge was used to generate lead formulations which were further optimized. The optimized formulation was then tested against Tricor® 145 in a single-dose, randomized, three-way crossover study in 18 healthy human volunteers.

2. Materials and methods

2.1. *Materials*

Crystalline fenofibrate was purchased from Solmag S.P.A (Italy) and used as received. Incromega E7010SR was purchased from Croda, Inc. (Edison, NJ). It contained 88.5 wt% total omega-

Table 1 – Lipid formulation types as proposed by Pouton (2000)

	Type I	Type II	Type IIIA	Type IIIB
Typical composition (%)				
Triglycerides or mixed glycerides	100	40–80	40–80	<20
Surfactants	–	20–60 (HLB < 12)	20–40 (HLB > 12)	20–50(HLB > 11)
Hydrophilic cosolvents	–	–	0–40	50–100
Particle size (nm)	Coarse	100–250	100–250	50–100
Significance of aqueous dispersion	Limited importance	Solvent capacity unaffected	Some loss of solvent capacity	Significant phase changes and potential loss of solvent capacity
Significance of digestibility	Crucial requirement	Not crucial but likely to occur	Not crucial but may be inhibited	Not required and not likely to occur

Table 2 – Excipients used in the HT experiment

Excipient	Category	Manufacturer	Additional description
Incromega E7010SR	Oil	Croda Inc.	Omega-3 oil with 70% EPA and 10% DHA
Vitamin E TPGS	Surfactant	Eastman	d-alpha-tocopheryl PEG 1000 succinate
Tween 85	Surfactant	Sigma	PEG(20) sorbitan trioleate
Tween 20	Surfactant	Sigma	PEG(20) sorbitan monolaurate
Tween 80	Surfactant	Sigma	PEG(20) sorbitan monooleate
Cremophor EL	Surfactant	BASF	PEG-35-castor oil
Labrasol	Surfactant/cosurfactant	Gattefosse	PEG-8 caprylic/capric glycerides
Poloxamer 331	Surfactant	BASF	
Gelucire 44/14	Oil/surfactant/cosurfactant	Gattefosse	Mono, di, and triglycerides with mono, diesters of PEG
Span 20	Cosurfactant	Sigma	Sorbitan monolaurate
Span 80	Cosurfactant	Sigma	Sorbitan monooleate
Labrafil M1944CS	Cosurfactant	Gattefosse	Apricot kernel oil PEG-6 esters
Labrafil M2125CS	Cosurfactant	Gattefosse	Corn oil PEG-6 esters
PEG 400	Cosolvent	Sigma	
Glycerol	Cosolvent	Sigma	
Propylene glycol	Cosolvent	Sigma	
Ethanol	Cosolvent	Sigma	
Transcutol P	Cosolvent	Gattefosse	Diethylene glycol monoethyl ether
α-Tocopherol	Additive	Sigma	

3, out of which 78.2 wt% is eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) in the form of ethyl esters. The ratio of EPA to DHA was 7:1 by weight. Ethyl alcohol 200 proof USP was purchased from Pharmco-Aaper (Shelbyville, KY). Natural hard gelatin capsules were purchased from Capsugel Inc. (Greenwood, SC). All other excipients used and sources are listed in Table 2. All materials were used as received.

2.2. *HPLC method*

HPLC was run using a gradient method on a Zorbax SB-C18, 5 μm, 250 mm × 4.6 mm column (Agilent Technologies, Santa Clara, CA). Column temperature was 40 ± 2 °C, and sample temperature was 25 ± 2 °C. The injection volume was 10 μL and the column flow rate was 1.2 ml/min. Mobile phase A was 0.05% trifluoroacetic acid in water, and mobile phase B was 0.05% trifluoroacetic acid in acetonitrile. The initial mobile phase was 40/60 vol% A/B. The linear gradient started at 1 min and ended at 15 min. The final mobile phase was 5/95 vol% A/B. The retention time of fenofibrate was 11.7 min in this method. UV quantitation of fenofibrate was performed at 288 nm.

2.3. *Ethanol effect on fenofibrate solubility*

The equilibrium solubility of fenofibrate was measured in mixtures of Incromega E7010SR and ethanol. Seven ratios of Incromega/ethanol solutions were tested: 100/0 (pure Incromega), 95/5, 90/10, 85/15, 80/20, 70/30, 60/40, 50/50, and 0/100 (pure ethanol) oil/ethanol (v/v) ratios. The solubility samples were prepared and incubated at 15 ± 1 °C. The solubility measurements were performed at 15 °C, which represents the lower temperature limit for a room temperature product label. Since the solubility of fenofibrate is highly dependent on temperature (data not shown), this temperature was chosen to assess the lower-bound solubility values.

2.4. *High-throughput (HT) screen for SEDDS*

2.4.1. *SEDDS screening process*

The objective of the HT screen was twofold. The first objective was to create SEDDS excipient mixtures and identify lead mixtures based on dispersion characteristics. The second objective was to study the behavior of each excipient and assess how it affected the mixture properties.

The HT screening process (Fig. 2) starts with the experimental design using proprietary software. Here, various combinations of excipient mixtures were specified. Details of the experimental design are described below. After the excipient combinations were designed, the combinatorial dispenser created the combinations in 1 ml glass vials arranged in a 96-well plate format. The contents were thoroughly mixed via a magnetic stir bar in each vial. The mixtures were then allowed to equilibrate at room temperature for 2 days.

After equilibration, the glass vials containing the excipient combinations were examined on a miscibility station for signs of phase separation. In this station, lasers scan the height of the vials and detect changes in solution refractive index which signify inhomogeneous (i.e. immiscible) phases. The miscibility of the solutions was also confirmed independently by visual examination. Immiscible combinations were recorded but remained in the experiment.

Dispersion behavior of each excipient combination was characterized in simulated fasted-state gastric fluid (SGF) (Dressman et al., 1998) at 37 °C. This process was achieved by transferring 8 μl of the excipient combinations into glass vials filled with 800 μl SGF incubated at 37 °C (approximately 100× dilution). The SGF was incubated at 37 °C to simulate actual gastric conditions. The vials were then capped and gently mixed with magnetic stir bars for 1 min. Immediately after mixing, 200 μl from the vials were transferred into 96-well polystyrene microtiter plates for optical density measurements (at 550 nm) and imaging.

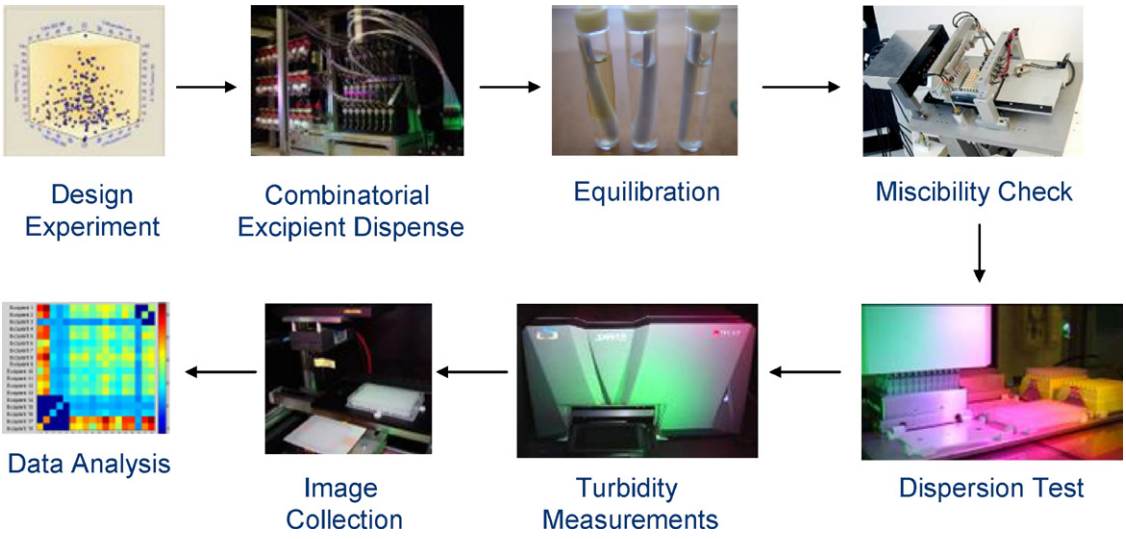


Fig. 2 – Schematic of the high-throughput screening process.

Data from the optical density measurements were analyzed using Spotfire™ (Spotfire Inc., Somerville, MA) data visualization software.

2.4.2. *Excipient selection and experimental design*

The excipients chosen for this experiment are listed in Table 2.

This experimental design generated a total of 768 unique excipient combinations ranging from binary to pentanary (five-component) mixtures. All of the combinations contained Incromega E7010SR, and most of the combinations contained ethanol at 15% (v/v). Different mixtures of surfactant, cosurfactant, and cosolvent were included to investigate their effects on the emulsification of the oil. However, the total emulsifier content was kept at 20% (v/v) or below to maximize the amount of omega-3 oil in the dosage.

After the mixtures were dispensed, equilibrated, and checked for miscibility, 463 out of 768 combinations were miscible while the remaining combinations showed various degrees of phase separation. Dispersion tests were performed on all combinations, regardless of miscibility.

2.5. *Optimization of SEDDS formulation containing fenofibrate*

After the HT screen data were analyzed, the results were used to create several prototype mixtures that were further manually optimized to yield the final formulation for human testing (formulation A). The optimization process involved selecting and refining the mixture composition such that a suitable balance of high fenofibrate solubility and good dispersion characteristics were obtained.

2.6. *Chemical stability of omega-3 fenofibrate formulation*

2.6.1. *Potential fenofibrate interaction with ethanol*

Fenofibrate, an isopropyl ester of fenofibric acid, can undergo transesterification with ethanol to form an ethyl ester under stress conditions. To establish the relative retention time (RRT) of this possible degradant using our HPLC method, fenofibrate was treated in excess ethanol (2 mg/ml drug solution) at reflux for 6 days using *p*-toluenesulfonic acid as a catalyst. The resulting sample was analyzed using the HPLC method described above.

2.6.2. *Chemical stability of formulation A*

Chemical stability of formulation A was performed over 17 weeks. Stability was assessed at 25 °C/60%RH, 30 °C/65%RH, and 40 °C/75%RH. The concentration of fenofibrate in the stability samples was 90.2 mg/ml. Samples for 25 °C and 30 °C incubation were filled into soft gelatin capsules (hydrophilic air-fills from Cardinal Health, Type L3DXHBHM) at a volume of either 790 μl or 804 μl (the variability came from the intrinsic variability in the capsule volume). The expected amount of fenofibrate per capsule was therefore 71 mg or 73 mg, respectively. These capsules were sealed in foil pouches (Technipag, Lot 6227-1) prior to incubation to minimize ethanol loss. Samples for 40 °C incubation were filled into 1.5 ml autosampler vials and crimp sealed. These vials were filled to the brim with sample to minimize the air gap in the vial. Fenofibrate content and chemical stability was monitored at selected time points using the HPLC method above.

2.7. *Human pharmacokinetic study*

Formulation A was evaluated in 18 healthy human subjects in a single-dose, randomized, three-way crossover study. The objective of the study was to evaluate the effect of the self-emulsifying formulation on the absorption of fenofibrate under both fasted and fed conditions as compared to the marketed Tricor® 145 mg tablet. Tricor® was only evaluated in our study under the fed condition since food effect studies have been published previously and no food effect was observed (http://www.rxabbott.com/pdf/tricorpi.pdf).

2.7.1. *Methods*

The study was conducted at the facilities of the Drug Research Laboratory, University of Tennessee, Memphis, Tennessee.

The study was a single-dose, randomized, 3-treatment, 3-sequence, 3-period crossover comparative bioavailability study. Eighteen subjects (nine males and nine females) were selected for this study, all of which completed the clinical phase. The subjects were divided randomly into three groups, each of which received the following treatments with a 7-day washout period between treatments:

(1) Formulation A, fed: fenofibrate/omega-3, 145 mg (2 × 72.5 mg capsules) after a standard breakfast.
(2) Formulation A, fasted: fenofibrate/omega-3, 145 mg (2 × 72.5 mg capsules) after an overnight fast.
(3) Tricor® 145, fed: Tricor® 145 mg tablet after a standard breakfast.

For each dosing period, the subjects reported to the clinical facility after a 10-h overnight fast. Standard FDA breakfast was administered to the randomly assigned fed subjects 30 min prior to dosing. The dose treatments were administered with 240 ml of water.

Venous blood samples were collected pre-dose (0 h) and 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 14 h, 24 h, 34 h, 48 h, and 72 h post-dose. Plasma from the collected blood samples were promptly separated and frozen until assayed by MDS Pharma Services (St. Laurent, Quebec) using a validated LC/MS assay for fenofibric acid (the active metabolite) in human plasma.

2.7.2. *Pharmacokinetic calculations*

Pharmacokinetic parameters, including AUC_{0-t}, AUC_{0-inf}, C_{max}, T_{max}, and $t_{1/2}$ were calculated from the individual concentration–time data for fenofibric acid using PhAST software (Version 2.3-004, Phoenix international Life Sciences Inc.). The highest experimental concentration was considered the peak concentration (C_{max}), time to C_{max} was denoted T_{max}. The observed terminal phase rate constant of elimination (K_{el}) was calculated from three terminal points or more, where possible, of the log linear regression. The number of points included in the calculation was selected to optimize the r^2 value of the regression analysis. The terminal phase half-life ($t_{1/2}$) was determined by dividing 0.693 by K_{el}. The area under the plasma concentration versus time curve from time zero to the last quantifiable concentration (AUC_{0-t}) was calculated by the linear trapezoidal method (Bailer, 1988). The area under the plasma concentration versus time curve from time zero to infinity (AUC_{0-inf}) was calculated as the sum of AUC_{0-t} plus the ratio of the last plasma concentration to K_{el} (Bailer, 1988).

Concentration values below the limit of quantification (20.1 ng/ml) were assigned a value of zero for pharmacokinetic analysis and descriptive statistics.

2.7.3. *Statistical analysis*

Analyses of variance (ANOVA) were performed on the ln-transformed AUC_{0-t}, AUC_{0-inf}, and C_{max}. Each ANOVA included calculation of LSM (least-square means), the difference between formulation LSM, and the standard error associated with this difference. Ratios of LSM were calculated using the exponentiation of the LSM from the analyses on the ln-transformed AUC_{0-t}, AUC_{0-inf}, and C_{max} pharmacokinetic parameters. These ratios were expressed as a percentage relative to the reference formulation (Tricor® 145).

3. Results

The overall goal of this study was to find a self-emulsifying formulation of fenofibrate that shows the best overall performance based on *in vitro* characterizations. Among the properties considered were omega-3 oil content, fenofibrate solubility in the formulation, self-emulsifying properties, and acceptability of excipients. Chemical stability of the resulting formulations was also assessed.

3.1. *Ethanol effect on fenofibrate solubility*

It was noticed that when ethanol was present with the omega-3 oil, solubility of fenofibrate in the mixture increased significantly. This synergistic increase in solubility was studied in greater detail in Incromega E7010SR at 15 °C. The results are shown in Fig. 3.

In pure oil, fenofibrate solubility was measured at 77 mg/ml. Upon addition of ethanol, the solubility increased to close to 120 mg/ml at approximately 20% ethanol. This illustrates a strong synergistic solubility increase in the presence of ethanol, as the solubility of fenofibrate in pure ethanol (0% Incromega) was only 33 mg/ml. This unique interaction between the oil, ethanol, and fenofibrate was utilized in the design of our SEDDS formulation to maximize fenofibrate solubility. The solubility behavior was not limited to omega-3 oil and similar synergistic increase was observed when several other lipids such as oleic acid, ethyl oleate, and propylene glycol dicaprylate (Captex 200) were tested instead of the omega-3 oil (data not shown).

3.2. *High-throughput screen results*

The first step in the HT screen was to identify surfactant(s) that provided the best emulsification. Qualitatively, as emulsification progresses from its initial unemulsified state, the turbidity of the sample monotonically increases until all of the oil phase has been emulsified. The turbidity of the solution will decrease if the emulsifier content is further increased due to the formation of opalescent microemulsions.

After visual examination of the excipient mixtures, it was determined that the maximum amount of emulsifiers being used in this experiment (20%, v/v) was insufficient for

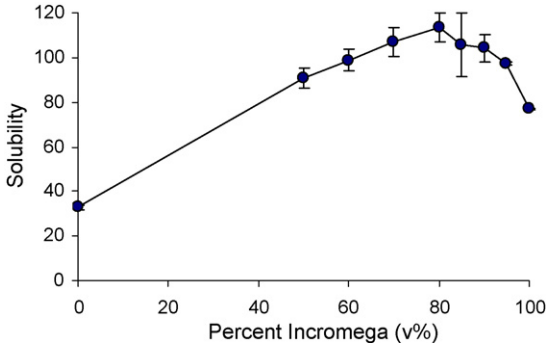


Fig. 3 – Equilibrium solubility of fenofibrate in Incromega E7010SR/ethanol mixtures at 15 °C ($n = 2$ for each data point).

microemulsions to form, as no microemulsions were observed in any of the mixtures. Therefore, the monotonic relationship between the degree of emulsification and the solution turbidity measured at 550 nm could be utilized to help rank the mixtures with respect to their emulsification efficiencies.

Table 3 compares images of the dispersions among the different types of emulsifiers. Comparisons of emulsifiers at 20% revealed that the best emulsifiers when used alone at this level were Cremophor EL, Tween 85, and vitamin E TPGS. The cosurfactants and cosolvents, as expected, did not yield good emulsification when used as the sole emulsifying component.

Samples containing the three surfactants that showed good emulsification behavior were re-examined at a 10 ml scale. At this scale, only Tween 85 showed complete emulsification. Although samples containing Cremophor EL or vitamin E TPGS were highly emulsified, small droplets of unemulsified oil were observed at the surfaces of both solutions.

Despite the good emulsification activity of Tween 85, it has not been extensively used in oral formulations of marketed drugs. While vitamin E TPGS showed good self-emulsifying activity, it was noticed that the rate of emulsification with TPGS was slower than with Cremophor EL due to the physicochemical properties of the mixture (vitamin E TPGS is a semi-solid at 37 °C). Therefore, optimization was focused around Cremophor EL as the main surfactant component.

Since one of the key formulation requirements was to restrict the emulsifier content to 20% or less in order to maximize the amount of Incromega E7010SR in the formulation, further increasing Cremophor EL content to improve emulsification was not pursued. Instead, mixtures of different types of emulsifiers were considered while maintaining the total emulsifier content at 20% (v/v). Some of the emulsifier mixtures considered were binary surfactant pairs, binary surfactant–cosurfactant pairs, binary surfactant–cosolvent pairs, and ternary surfactant–cosurfactant–cosolvent mixtures. The optical density values were analyzed using Spotfire™ data visualization package to help study the effects of the mixtures on the emulsification.

Based on the high-throughput screen results, a number of Cremophor EL-containing mixtures yielded turbidity values matching, if not exceeding, that of Cremophor EL when used as the sole emulsifier. An example is shown in Fig. 4. Each data point in the plot is a unique mixture containing 65% Incromega E7010SR, 15% ethanol, and 20% emulsifier mixture (all vol%). The emulsifier mixture contained binary mixtures of Cremophor EL with different cosurfactants. The volume percentage of Cremophor EL relative to the cosurfactant is shown on the x-axis.

At 0% Cremophor EL, the mixtures contain only the cosurfactant at 20%. With the exception of Span 20 and Span 80, diluting Cremophor EL with cosurfactants showed a negative impact on the emulsification. With the Spans, however, the results were different. As the Span content increased, the turbidity of the mixture increased and reached a maximum at 50% before decreasing back to the pure Cremophor EL values.

The turbidity results were confirmed when the 50/50 mix between Cremophor EL and either Span 20 or Span 80 was investigated further through scaling-up to a 10 ml volume. The results showed that the mixture containing 65% Incromega E7010SR, 15% ethanol, and 20% of a 50/50 mixture of Cremophor EL and Span 80 resulted in rapid emulsification in SGF. However, very small droplets of unemulsified oil can still be seen among the coarse emulsion particles. In comparison, when Span 20 was used at the same ratio, complete emulsification was observed. After a number of optimization studies, it was determined that this mixture yielded the best self-emulsifying properties and was suitable for a clinical formulation (Table 4).

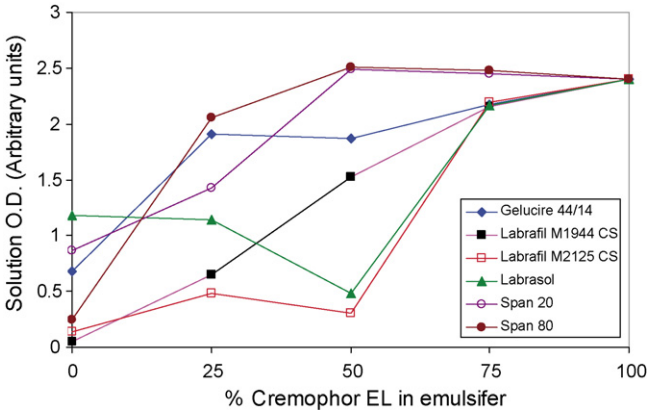


Fig. 4 – Turbidity of mixtures containing 65% Incromega E7010SR, 15% ethanol, and 20% of a binary emulsifier mixture. The x-axis shows the percentage of Cremophor EL in the binary emulsifier mixture.

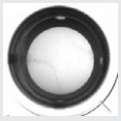
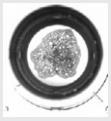
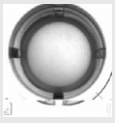
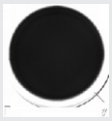
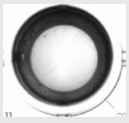
3.3. *Formulation A—the clinical formulation*

Formulation A was made by dissolving fenofibrate into the optimized self-emulsifying excipient mixture shown in Table 4 at 90.24 mg/ml. With this formulation, 145 mg of fenofibrate can be delivered with two soft-gelatin capsules each containing approximately 800 μl of formulation A. On a weight basis, the composition of formulation A is shown below (Table 5).

Since the HT screen was designed to look for compositions that showed the best self-emulsifying characteristic by evaluating a large mixture space, a significant number of excipient mixtures were tested. Fenofibrate was not added to the excipient mixtures to conserve material. Therefore, the dispersion tests in the HT screen were carried out with excipient mixtures in the absence of the drug. As a result, the effect of fenofibrate on the dispersion characteristics of formulation A needed to be examined. Indeed, a dispersion test of formulation A showed a slight decrease in emulsification in the presence of fenofibrate. However, the majority of the formulation still self-emulsified and only a very small amount of oil remained visible as minute droplets in solution. To achieve the same degree of self-emulsification seen with the placebo mixture would require increasing the amount of emulsifiers used. This would decrease the amount of omega-3 oil in the formulation and was not desirable.

A dispersed sample of formulation A is shown in Fig. 5 at a 20 ml scale. Dynamic light scattering measurements reported an average droplet size of approximately 200 nm.

Table 3 – Ability of different emulsifiers to emulsify Incromega, based on appearance (imaging) and optical density (O.D.) measurements at 550 nm

Emulsifier	Dispersion appearance	O.D. at 550 nm	Comments
Crillet 4HP (Tween 80)		2.041	Partially emulsified
Tween 20		0.568	Partially emulsified
Tween 85		2.494	Highly emulsified
Cremophor EL		2.399	Highly emulsified
Poloxamer 331		0.370	Partially emulsified
Labrasol		1.178	Partially emulsified
Vitamin E TPGS		2.510	Highly emulsified
Span 20		0.8702	Partially emulsified
Span 80		0.243	Not emulsified
Labrafil M1944CS		0.050	Not emulsified
Labrafil M2125CS		0.140	Not emulsified
Gelucire 44/14		0.677	Partially emulsified

Each sample well contained Incromega E7010SR/ethanol/emulsifer at 65/15/20 v/v/vol%.

Table 4 – Composition of the optimized self-emulsifying excipient mixture

Components	v/v (%)
Incromega E7010SR	65
Ethanol	15
Cremophor EL	10
Span 20	10

3.4. *Chemical stability of omega-3 fenofibrate formulation*

3.4.1. *Potential fenofibrate interaction with ethanol*

HPLC analysis showed a very small amount of ethyl ester product after 6 days of reflux. The amount was not quantified, but rather the presence of the peak was used to identify the location of the putative degradant in HPLC traces. The identity of the ethyl ester form was confirmed using mass spectroscopy. The relative retention time of the ethyl ester of fenofibric acid is 0.96 RRT in the HPLC method used (where fenofibrate, the isopropyl ester, has the RRT of 1.0).

3.4.2. *Chemical stability of formulation A*

Formulation A was observed to be physically stable at 15 °C which is consistent with a room temperature storage label for this product (i.e., 15–30 °C). Fenofibrate did not lose potency or degrade chemically in a detectable manner under the applied stress conditions over the 17-week study period, as shown in Table 6. Fenofibrate related degradants were not observed nor was the ethyl ester form of fenofibric acid.

3.5. *Pharmacokinetic results*

A summary of the relevant pharmacokinetic parameters is shown in Tables 7 and 8. The mean plasma concentrations of fenofibric acid versus time curves (linear and semi-log scale) are shown in Fig. 6A and B.

When formulation A was administered in the fasted state, the pharmacokinetic parameters were comparable to those of Tricor® 145 after a standard breakfast. No significant differences between the formulations were observed in mean AUC, C_{max}, T_{max}, and half-life values (Tables 7 and 8).

Interestingly, when formulation A was administered in the fed state, absorption of fenofibrate appears to be delayed as compare to Tricor® 145 as suggested by the observed T_{max} (mean values of 6.56 h and 2.95 h, respectively). C_{max} was also significantly lower when formulation A was administered in the fed state. However, ANOVA analysis suggests that AUC values are statistically equivalent. Based on AUC values alone, formulation A can be considered equivalent to Tricor® 145 in the fed state.

The observation that the absorption of formulation A was delayed in the fed state is interesting. It may be attributed to the greater inter-mixing of the SEDDS formulation with the food/fat content in the stomach which slowed down its gastric emptying as compared to the tablet.

Table 5 – Composition of the clinical formulation (formulation A)

Components	wt%
Incromega E7010SR	63.4
Ethanol	9
Cremophor EL	9
Span 20	9
Fenofibrate	9.6

Fig. 5 – Coarse emulsion obtained by dispersing formulation A into simulated gastric fluid at 300× dilution, 37 °C. This corresponds approximately to a 0.8 ml capsule in 250 ml SGF.

4. Discussion

There are several "rule-of thumb" guidelines, many of which are based largely on empirical observations, to help formulate self-emulsifying lipid formulations (Holmberg et al., 2003). One of which utilizes the HLB (hydrophilic–lipophilic balance) of the ingredients to determine the mixture ratio that gives maximum emulsion stability. It has been empirically found that a combination of emulsifiers, one with high HLB and the other with lower HLB, often yields emulsions with greater physical stability than just one surfactant with an intermediate HLB value. A common hypothesis behind this observation is that a mixture of two different surfactants allows more efficient packing at the oil–water interface (Holmberg et al., 2003).

When a mixture of emulsifiers is used, the ratios in which the emulsifiers are combined also affect the emulsion stability. It was found that emulsions with the best stability can generally be obtained when the HLB of the emulsifier (or average HLB in the case of mixtures) is equal to that of the oil (Rosen, 1989).

Table 6 – Fenofibrate potency and chemical stability over 17 weeks as determined by HPLC

Storage container	Storage condition	Time point (weeks)	Fenofibrate amount per capsule (mg)	Fenofibrate percent area composition (LC area % 285 nm)
Gelatin capsule	Initial	0	73	100
Gelatin capsule	25 °C/60%RH	4	71	100
Gelatin capsule	25 °C/60%RH	17	73	100
Gelatin capsule	30 °C/65%RH	4	73	100
Gelatin capsule	30 °C/65%RH	17	73	100
1.5 ml crimp vial	40 °C/75%RH	2	74[a]	100
1.5 ml crimp vial	40 °C/75%RH	4	84[a]	100
1.5 ml crimp vial	40 °C/75%RH	17	85[a]	100

[a] Expected fenofibrate amount was 73 mg. Deviations from the expected value were due to sampling variability from glass vials. No such variability was seen for the capsules since the entire capsule content was analyzed and no sampling performed.

Table 7 – Summary of mean (standard deviation) pharmacokinetic parameters of fenofibric acid in humans following oral administration of fenofibrate formulations

Treatment	AUC_{0-t} (ng h/ml)	AUC_{0-inf} (ng h/ml)	C_{max} (ng/ml)	Half-life (h)	T_{max} (h)
Tricor® 145 mg fed	171342 (41293.4)	176291 (44249.0)	10828.8 (2035.8)	20.0 (4.3)	2.95 (1.1)
Formulation A fed	184214 (38500.7)	189561 (41134.7)	8255.7 (2476.7)	19.4 (4.4)	6.56 (4.7)
Formulation A fasted	168512 (46509.3)	178761 (48623.0)	10453.5 (1892.1)	21.2 (4.2)	2.12 (0.7)

Table 8 – Ratio and 90% confidence intervals of pharmacokinetic parameters

Treatments	Log transformed AUC_{0-t}	Log transformed AUC_{0-inf}	Log transformed C_{max}
Formulation A fed/formulation A fasted	109.3% (104.5–114.4%)	107.5% (102.7–112.5%)	79.0% (72.6–85.9%)
Formulation A fed/Tricor® fed	107.5% (102.7–112.5%)	107.6% (102.9–112.5%)	76.2% (70.1–82.9%)

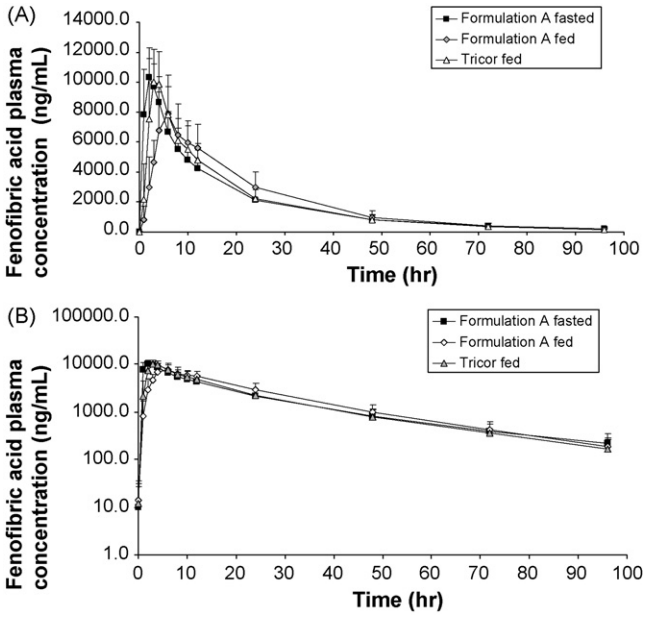


Fig. 6 – (A) Mean plasma concentration–time curves (linear scale) for formulation A and Tricor® tablets after oral administration to healthy volunteers ($n = 18$). (B) Mean plasma concentration–time curves (semi-log scale) for formulation A and Tricor® tablets after oral administration to healthy volunteers ($n = 18$).

Although this provides a general guideline for the formulation of lipid emulsions, access to a high-throughput experimentation platform allowed us to explore a wide combinatorial space not practical for benchtop experiments. This has also allowed us to verify the empirical guidelines by creating mixtures using emulsifiers with various HLB values and screening them for ones that offered the best emulsifying characteristics.

Based on information from the manufacturer, the experimental HLB value of Incromega E7010SR is 10.5. The HLB values of selected relevant excipients and combinations are shown in Table 9.

In our study, the emulsifier mixture containing 50:50 Cremophor EL:Span 20 (used in formulation A), was shown to give very good emulsion stability, and it did indeed have an HLB value almost the same to that of the oil.

Once verified, the empirical guidelines can now be used in combination with the high-throughput approach to better design future experiments.

Table 9 – HLB values of selected emulsifiers

Emulsifier	Approximate HLB
Cremophor EL	13
Span 80	4.3
Span 20	8.6
Cremophor EL:Span 80 (50:50)	8.6
Cremophor EL:Span 20 (50:50) (formulation A)	10.8

It is necessary to point out that in clinical studies for Tricor®, an elevation of low-density lipoprotein (LDL) cholesterol was observed in patients with hypertriglyceridemia (Fredrickson Types IV and V). While the exact mechanism and clinical relevance of these LDL increases were not understood, it was hypothesized that these changes were a result of a shift from smaller/denser and more atherogenic particles to more buoyant and less atherogenic ones (http://www.fda.gov/cder/foi/nda/2000/19304-S005_Tricor.htm). High dose (4 g) of omega-3 oil can also result in an increase in LDL cholesterol. However, this effect was absent when a lower dose (1 g) was studied (http://www.fda.gov/cder/foi/nda/2004/21-654_Omacor.htm). Nevertheless, monitoring of LDL cholesterol would be necessary in any future clinical studies of the fenofibrate/omega-3 combination.

5. Conclusion

A self-emulsifying formulation of fenofibrate was developed using high-throughput experimentation and tested in healthy human volunteers. The use of a high-throughput screening platform allowed more than 700 possible formulations to be evaluated in less than 2 weeks, and proved to be a valuable tool in the identification of self-emulsifying mixtures. The screen yielded a number of lead formulations that were further optimized and refined to give optimum *in vitro* self-emulsifying characteristics in addition to high fenofibrate solubility and good chemical stability.

The resulting formulation (formulation A) consisted of omega-3 oil, ethanol, and Cremophor EL and Span 20. In simulated gastric fluid at 37 °C, the formulation quickly emulsified into fine emulsion droplets of approximately 200 nm in size with mild agitation. The self-emulsifying formulation was tested in human volunteers under both fed and fasted conditions, and compared to the marketed fenofibrate tablet, Tricor®, administered in the fed state. The AUC_{0-t} and AUC_{0-inf} for formulation A were both within the 80–125% FDA acceptance range of Tricor®. No food effect was observed when AUCs were compared from the fed and fasted states. However, absorption was slightly slower in the fed state, possibly due to slower gastric emptying of the dispersion in the post-prandial state.

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