Research Article

FORMULATION AND *IN-VITRO* EVALUATION OF FENOFIBRATE DRY EMULSION IN HARD VEGETARIAN CAPSULES

Madadi Sunitha Reddy *, Mallika Banu

Center for Pharmaceutical Sciences, IST, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana, India

*Corresponding Author Email: baddam_sunitha@rediffmail.com

Article Received on: 10/11/17 Approved for publication: 17/12/17

DOI: 10.7897/2230-8407.0812248

ABSTRACT

The dry emulsion formulation aims to improve the solubility and dissolution of Fenofibrate. Dry emulsions are an attractive choice because their physical and micro biological stability. They represent a potential oral drug delivery system for lipophilic and low soluble drug substances. Dry emulsions can be prepared by spray drying, lyophilization or rotary evaporation. Dry emulsion prepared here was by lyophilization using flaxseed oil as the lipid phase and HPMC as the carrier. Fenofibrate was the drug of choice as it helps reduce cholesterol and triglycerides (fatty acids) in the blood. Flaxseed oil has been studied for lowering triglycerides. Hence the combination therapy of Fenofibrate and Flaxseed oil in low doses is expected to have a synergistic activity in lowering the cholesterol and triglycerides in the blood. The dry emulsion formed has been incorporated in HPMC (Hydroxyl Propyl Methyl Cellulose) capsules, which provides a vegetarian environment and is a good alternative to gelatin. The formed dry emulsion formulation has been evaluated for the respective parameters in comparison with the marketed variant and the results are obtained.

Keywords: HPMC, Fenofibrate, Dry Emulsion, Vegetarian environment, Flax seed oil, Antihypertensive.

INTRODUCTION

High blood pressure is a condition where the long-term force of blood against the artery walls is so high that it causes health problems such as strokes, heart attacks¹, heart failure, or kidney disease. The aim of hypertension treatment is to lower high blood pressure and protect important organs, like the brain, heart, and kidneys from damage.

More than 10 million cases per year have been recorded in India

Table 1: Incidence rate of hypertension by age²

AGE IN YEARS	INCIDENCE RATE
>60	66.7 %
40 - 59	30.4 %
18 - 39	6.8 %

Blood pressure stages³

Table 2: Different stages of blood pressure

Blood Pressure Category	Systolic mm Hg (Upper limit)		Diastolic mm Hg (Upper limit)
Low Blood Pressure (Hypotension)	Less than 80	or	Less than 60
Normal	80 - 120	and	60 - 80
Prehypertension	120 - 139	or	80 - 89
High Blood Pressure (Hypertension Stage 1)	140 - 159	or	90 - 99
High Blood Pressure (Hypertension Stage 2)	160 or higher	or	100 or higher
High Blood Pressure Crisis (Seek Emergency Care)	Higher than 180	or	Higher than 110

FENOFIBRATE is a drug of the fibrate class. It is mainly used to reduce cholesterol levels in people at risk of cardiovascular disease. Like other fibrates, it reduces low-density lipoprotein

(LDL) and very low density lipoprotein (VLDL) levels, as well as increasing high-density lipoprotein (HDL) levels and reducing triglyceride levels⁴.

Figure 1: Mechanism of action of Fenofibrate⁵

Fenofibric acid is the metabolite of fenofibrate, which produces reduction in LDL, total triglycerides and VLDL in patients. Treatment with fenofibrate results in increased HDL and apoproteins apo AI and apo AII⁶. Fenofibrate is used for primary hypercholesterolemia or mixed dyslipidemia. Fenofibrate reduces the risk of cardiovascular disease and diabetic retinopathy in patients with diabetes mellitus, and indicated for reducing the progression of diabetic retinopathy in patients with type 2 diabetes. It is helpful in decreasing amputations of the lower legs⁷.

Flaxseed oil is rich in omega-3, omega-6, α -linolenic acid, lignans, high quality proteins and fibers, which are biologically active in the prevention of chronic diseases such as cancer, diabetes, cardiovascular diseases and stroke⁸. Omega-3 deficiency is associated with lower intelligence, depression, heart disease, arthritis, cancer and other health problems.

Gelatin is a protein made from animal products like tendons, ligaments. It is a protein made from the skins and bones of pigs and cows. It's a common ingredient in a number of products, including:

- Makeup products
- Medicine formulations, vitamins
- Food products, drugs
- Vaccines preparations

Problems with gelatin⁹

1. Allergy, Dietary Restrictions, toxins exposure
2. Cannot incorporate highly moisture sensitive drugs and deliquescent materials
3. Cannot incorporate efflorescent material
4. Difficult to incorporate water soluble materials

Search for gelatin replacement lead to absence of bovine spongiform encephalopathy (BSE), cross linking of gelatin, abnormal liver and kidney function, strict regulations regarding the use of animal derived gelatin, drug incompatibilities.

Hence HPMC, which is one of the alternatives to gelatin in pharmaceutical preparations, has been chosen for the study. Another reported advantage of HPMC capsules over gelatin capsules is related to the difference in moisture content of the

shells. Because HPMC shells contain significantly less moisture compared to hard gelatin capsules by almost one third, it is compatible with hygroscopic materials¹⁰.

Oral Emulsions are liquids with one or more active pharmaceutical ingredients and are stable oil-in-water dispersions, where one or both phases may contain dissolved solids. Liquid emulsions have distinct advantages as they improve the bio availability and reduce the side effects of drug but lack physical, chemical and compliance problems¹¹.

To overcome these problems dry emulsions are prepared. Dry emulsions are lipid based powder formulations from which an O/W emulsion can be reconstituted In-vivo or In-vitro. They are prepared by drying liquid O/W emulsions containing a solid carrier in the aqueous phase. A solid carrier provides them with bulk and mass.

General method of preparation of dry emulsion follows the steps given below

1. Drug is dissolved in lipophilic solvent.
2. Aqueous phase is added containing bulking agents
3. Emulsion is formed
4. Water is removed (Lyophilization, spray drying)
5. Powder is filled into capsules or tablets are compressed

Dry emulsions are prepared by using

- Spray drying
- Freeze drying/Lyophilization
- Rotary evaporation

The method chosen to prepare the Fenofibrate dry emulsion was Lyophilization or Freeze drying. The lyophilization procedure is used to preserve a liquid, creamy or a solid product by withdrawing the water through sublimation under vacuum, the water changes directly into the gaseous state (steam). The cooling coil releases the steam which is caught up in and removed.

The different phases of lyophilization¹²

The process of freezing: The products should be frozen, the water is turned into ice.

The primary drying: The intracellular water is sublimed and then water gets evaporated, which is caught up and re-solidified on cold condenser plates at -60 to -70°C .

The secondary drying: The products are heated up. The maximum temperature is $+50^{\circ}\text{C}$.

Freeze-dried products are produced with residual moisture of about 1 – 5%.

Two phases are extremely important during the lyophilization

The freezing: water gets transformed into ice very fast in this phase and not in form of small ice crystals.

The vacuum: The most important step for the lyophilization is the quality of vacuum. The freeze-dryer has to be absolutely leak proof (structure, valves, etc.). The vacuum pumps must have enough power to allow a perfect vacuum.

MATERIALS AND METHODS

Drug:	Fenofibrate
Surfactant & Co-surfactant Mix:	Span 80 & Tween 80 (1:4)
Lipid:	Flaxseed oil
Carrier:	HPMC
UV Spectrophotometer:	Shimadzu 1800
IR Spectrophotometer:	Bruker Alpha
Dissolution Apparatus:	DS8000 lab India

Method development and validation of UV-spectroscopic method for the determination of Fenofibrate:

Preparation of standard stock solution

Standard solution of Fenofibrate was prepared by taking 10 mg of the drug and dissolving it in 10 ml of 5% methanol v/v in 0.1 N HCl (1000ppm).

Preparation of concentrations of analytical range

The working standard solution of the drug is prepared by pipetting out 1ml of the solution into 10 ml of the volumetric flask and made up to the volume with 5% methanol v/v in 0.1 N HCl (100ppm). Then, respective volumes of solutions were pipetted out from the 100ppm solution into 10ml volume flasks to obtain 2, 4, 6, 8, 10 ppm of Fenofibrate.

Determination of λ_{max} of Fenofibrate in 5% methanol v/v in 0.1 N HCl

- 2, 4, 6, 8, 10ppm of Fenofibrate solutions were prepared in 5% methanol v/v in 0.1 N HCl.
- UV scan of the above solution was taken between 400-200 nm.
- The spectrum shows a maximum absorption at **272nm**.

Standard graph of Fenofibrate in 5% methanol in 0.1 N HCl

- A Standard stock solution of Fenofibrate was prepared by dissolving 100mg of drug in 100 ml of 5% methanol v/v in 0.1 N HCl
- From the standard stock solution, concentrations of 2, 4, 6, 8, 10ppm were prepared using 5% methanol v/v in 0.1 N HCl
- Each solution was analyzed by UV Spectrometer at 272nm. The absorption values were noted.

Compatibility studies

- FT-IR spectrum of pure drug and drug-excipient were obtained by FT-IR spectrophotometer
- FT-IR spectrum of pure drug and drug-excipient were almost similar because of same functional groups. It indicates there was no interaction between Fenofibrate and excipients used in formulation.
- **FT-IR procedure:** FT-IR spectrum of pure drug, Fenofibrate and formulation were obtained by FT-IR spectrophotometer. The spectra were taken with the accumulations 24 scans and a resolution of 4cm^{-1} over the range of $400\text{--}4000\text{ cm}^{-1}$. The spectrum of formulation so obtained was compared with the spectrum of pure drug for any interactions.

Solubility studies

Solubility of drug in oil (flaxseed oil) was carried by placing an excess amount of Fenofibrate in vial containing 1ml of Flax seed oil and heated on a water bath at 35°C to facilitate the solubilization using vortex mixer. The vial was then continuously agitated on a rotary shaking incubator for 48hours at ambient temperature. After reaching equilibrium the sample were centrifuged at 3000 rpm for 10min and the supernatant was taken by pipette. The sample was suitably diluted with 5% methanol v/v in 0.1 N HCl and analyzed spectrophotometrically for the dissolved drug at 272nm. 5% methanol v/v in 0.1 N HCl was used as blank. Solubility studies were carried by placing an excess amount of Fenofibrate in vials containing 1gram of excipients (oil, surfactants and co-surfactants). The rest of the solubilizing procedure was same as that done for the oil. After reaching equilibrium the samples were centrifuged at 3000 rpm for 10min and the supernatant was taken by pipette. The samples were suitably diluted with 5% methanol v/v in 0.1 N HCl and analyzed spectrophotometrically for the dissolved drug at 272nm. Blank was prepared by dissolving respective vehicles in 5% methanol v/v in 0.1 N HCl with same dilution as for the samples. (See figure 2)

Droplet size & zeta potential

1 ml of samples of products with drug, oil and surfactant combinations were taken in 10 ml volumetric flask and were diluted with milli Q water up to the mark. These samples were then analyzed for particle size and zeta potential using Malvern instrument (Zeta sizer).

Lyophilization process

Two 10ml vials were dried and each filled with Span80 & Tween80 in the ratios 1:4 respectively with oil in the ratio 1:9 and 67mgdrug. 1gram5% HPMC of grade 5cps in milli Q water was mixed in Vial 1 and labelled as F1, 3 grams of 5% HPMC of grade 5cps in milli Q water was mixed in Vial 2 and labelled as F2 and are partially stoppered and transported to the lyophilizer. Each formulation was lyophilized separately to minimize possible interactions. After completion of lyophilization, vacuum was released and vials were automatically stoppered by the lyophilizer which were then sealed and stored for further evaluation studies.

Formulation in F2 vial has been dried successfully and the formulation in F1 vial still was in liquid state. Hence the formulation F2 was selected for further studies. (See figure 3, a, b).

In-vitro dissolution

Medium: 0.05M Sodium Lauryl Sulphate in 0.1 N HCl

Apparatus: Paddle (USP Type I)

Time: 40 minutes

Temperature: 37 +/- 1 °C

Rate: 75 RPM

Drug: 80mg

Dissolution rate of pure Fenofibrate and dry emulsion of Fenofibrate which are filled in HPMC capsules were carried out using the basket apparatus (USP Type I) at 37 °C in 900 ml of 0.1 N HCl at 75 rpm. Samples equivalent to 67 mg of Fenofibrate were subjected to the testing. At the specific time intervals 5 ml samples of dissolution medium were withdrawn, filtered and analyzed at 272 nm using UV spectrophotometer. At each time of withdrawal, 5ml of fresh 0.1 N HCl was added in the dissolution tank.

Accelerated stability studies

Accelerated stability studies of formulation were carried out as per ICH guidelines by storing the formulation at 40°C±2°C and RH 75%±5% for 1 month in stability chamber and later after 1-

month formulation was evaluated for parameters such as particle size, PDI and In-vitro drug release.

RESULTS

Standard graph of fenofibrate

Standard Table Report

06/20/2017 11:06:35 AM

File Name: C:\Documents and Settings\admin\My Documents\fenofibrate stnd graph.pho

Figure 4: Standard graph of Fenofibrate

Table 3: Standard graph table

	Sample ID	Type	Concentration	Wave Length	Wgt.Factor
1	Feno 2ppm	Standard	2.000	0.170	1.000
2	Feno 4ppm	Standard	4.000	0.318	1.000
3	Feno 6ppm	Standard	6.000	0.444	1.000
4	Feno 8ppm	Standard	8.000	0.569	1.000
5	Feno 10ppm	Standard	10.000	0.691	1.000

FT-IR studies

Figure 5: FT-IR spectrum of Fenofibrate drug

Figure 6: (i)= Fenofibrate + Span 80,(ii)= Span 80

Figure 7: (iii)= Fenofibrate + Tween 80,(iv)= Tween 80

Figure 8: (v) = Fenofibrate + HPMC,(vi)= HPMC

Solubility profile

Table 4: Solubility of drug in various excipient combination ratios

SMIX	SMIX ratio	OIL	SMIX & OIL ratio	Concentration
Span 80+Tween 80	1:4	Flax seed oil	1:9	87mg/ml+/-0.0099
Span 80+Tween 80	1:4	Flax seed oil	2:8	60mg/m+/-0.0100
Tween 80+Propylene Glycol	1:4	Flax seed oil	2:8	50mg/m+/-0.0152
Tween 80+Propylene Glycol	1:4	Flax seed oil	3:7	35mg/m+/-0.0100
Tween 80+Propylene Glycol	1:4	Flax seed oil	1:9	31mg/ml+/-0.0099

Droplet size measurements

Figure 9: Span 80+Tween 80 (1:4) + Fenofibrate + Flaxseedoil (1:9)

Zeta potential

Figure 10: Span 80 +Tween 80 (1:4) + Fenofibrate + Flaxseed oil (1:9)

In-vitro dissolution

Table 5: Cumulative % drug release of marketed drug and prepared formulation.

Time in minutes	% Drug release of marketed product	% Standard deviation	% Drug release of prepared formulation	% Standard deviation
5	20.42	0.0099	24.37	0.0099
10	25.51	0.0099	30.41	0.0100
20	37.72	0.0152	38.28	0.0099
30	42.36	0.0152	40.32	0.0152
35	56.41	0.0100	58.28	0.0099
40	60.88	0.0100	63.31	0.0100

Where n=3

Figure 11: Comparison of % drug release

Accelerated Stability Studies

Table 6: Results of Droplet size, PDI, % Drug release

Formulation	Droplet size (d.nm)	Polydispersity Index (PDI)	% Drug Release
F2	192.5	0.401	61.52 +/- 0.0152

Figure 12: Droplet size distribution of Dry emulsion formulation

Table 7: Cumulative % Drug release from Dry emulsion of Fenofibrate in 0.1 N HCl at the end of 1 month accelerated stability study

Time in minutes	% drug release of Dry emulsion
5	26.10+/-0.0152
10	35.24+/-0.0100
20	41.03+/-0.0208
30	43.5+/-0.0099
35	52.15+/-0.0099
40	61.51+/-0.0100

Figure 13: In-Vitro Dissolution profile of Fenofibrate dry emulsion at the end of 1 month accelerated stability study.

Figure 2: Solubility of drug & oil in different excipients.

Figure 3: (a) = F2 vial after lyophilization.
(b) = F1 vial after lyophilization.

DISCUSSION

Interpretation of FT-IR peaks

Table 8: Comparison of IR spectrum of pure fenofibrate and drug with excipients.

Groups	Fenofibrate	Feno+HPMC	Feno+Span 80	Feno+Tween 80
C=O	1650	1634.4	1656	1643
C-N	1287	1231	1243	1299
C-O	1012	1056	1089	1110
C=C	1729	1634	1744	1737
C-CL	764.3	690	725	764

There were no major changes seen in the FT-IR spectra of the pure drug in comparison to the drug and excipient combinations.

Solubility profile

The solubility of the drug in various excipient ratios and combinations were seen and it was found that the following combination showed the highest solubility among the rest:

Table 9: Solubility of fenofibrate in selected combination

SMIX	SMIX ratio	OIL	SMIX & OIL ratio	Concentration
Span 80+Tween 80	1:4	Flax seed oil	1:9	87mg/ml

Droplet size & Zeta potential

The zeta potential and the droplet size analysis of the drug along with the oil and surfactant mix was done and the desired combination of surfactant mix and oil was found to be Span80 & Tween80 in the ratios 1:4 respectively with oil in the ratio 1:9.

Lyophilization

Fenofibrate dry emulsion was prepared by lyophilization using Flaxseed oil as the lipid phase and Span 80, Tween 80 as the surfactant and co-surfactant and HPMC as the carrier. The dry emulsion obtained was filled in HPMC capsules and was compared with the marketed formulation.

Accelerated stability studies

Accelerated stability studies at 40°C and 75% RH for dry emulsion of Fenofibrate was performed and the droplet size is 192d.nm and PDI was found to be 0.401. Cumulative % drug release of Fenofibrate was found to be 61.52% at the end of 1 month indicating no change in % drug release after stability study for 1 month.

ACKNOWLEDGMENT

I would like to convey my sincere thanks to Centre for Pharmaceutical Sciences, IST, JNTUH for excellent laboratory facilities necessary for carrying out this work. I would like to

thank Gattefosse, Mumbai for providing gift samples of vehicles. Gift sample of Fenofibrate was obtained from laboratories and duly acknowledged

REFERENCES

1. mayoclinic.org [homepage on the Internet]. [Updated Sept. 09, 2016, Cited: May, 07, 2017] Available from: <https://www.mayoclinic.org/diseases-conditions/high-blood-pressure/basics/causes/con-20019580?p=1>
2. cdc.gov[homepage on the Internet]. [Last updated: October 2, 2012, Cited: May, 28, 2017] Available from: <https://www.cdc.gov/nchs/products/databriefs/db107.htm>
3. heart.org[homepage on the Internet]. [Updated: Oct 23,2017, Cited: May, 07, 2017] Available from: https://www.heart.org/HEARTORG/Conditions/HighBloodPressure/GettheFactsAboutHighBloodPressure/The-Facts-About-High-Blood-Pressure_UCM_002050_Article.jsp
4. wikipedia.org[homepage on the Internet]. [Updated: 21 October 2017, Cited: Feb, 03, 2017]Available from: <https://en.wikipedia.org/wiki/Fenofibrate>
5. rxlist.com[homepage on the Internet]. [Updated: 2/27/2017, Cited: June, 15, 2017] Available from: <https://www.rxlist.com/tricor-drug.htm>
6. wikipedia.org[homepage on the Internet]. [Updated:21 October 2017, Cited: Feb, 07, 2017]Available from: https://en.wikipedia.org/wiki/Fenofibrate#Mechanism_of_action

7. umm.edu [homepage on the Internet]. [Updated:6/22/2015, Cited: May, 28, 2017] Available from:<http://www.umm.edu/health/medical/altmed/supplement/flaxseed-oil>
8. webmd.com[homepage on the Internet].Available from:<https://www.webmd.com/vitamins-supplements/ingredientmono-1051-gelatin.aspx?activeingredientid=1051>
9. Moawia M. Al-Tabakha: HPMC Capsules: Current Status and Future Prospects; J Pharm Pharmaceut Sci (www.cspsCanada.org) [serial on the Internet] 13(3) 428 - 442, 2010, Cited: Feb, 03, 2017. Available from: <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.849.5631&rep=rep1&type=pdf>
10. Haritha M*, Priyanka M, Abeda Aqther, Neeharika R, Pragati Kumar B, Dry emulsion: A promising dosage form to deliver lipophilic drug molecules with improved stability and effectiveness, Indian Journal of Research in Pharmacy and Biotechnology [serial on the Internet] ISSN: 2320 – 3471 Volume 1 Issue 1 Page 119 10 121. Cited: May, 07, 2017. Available from:<https://pdfs.semanticscholar.org/4ddd/fa874490b3fa640e4c1436660d0e1bc69f23.pdf>
11. lyopharm.it [homepage on the Internet].Available from: <http://www.lyopharm.it/en/process-of-lyophilization.htm> Cited: May, 07, 2017

Cite this article as:

Madadi Sunitha Reddy and Mallika Banu. Formulation and In-vitro evaluation of fenofibrate dry emulsion in hard vegetarian capsules. *Int. Res. J. Pharm.* 2017;8(12):38-45 <http://dx.doi.org/10.7897/2230-8407.0812248>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.