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Validated spectrofluorometric method for determination of gemfibrozil in self nanoemulsifying drug delivery systems (SNEDDS)

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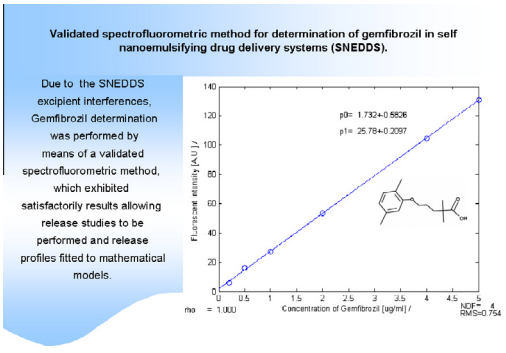
HIGHLIGHTS

- A spectrofluorometric method has been developed for the determination of gemfibrozil.
- The method is based on the excitation and emission capacities of gemfibrozil.
- This method allows de determination of the drug in SNEDDS.
- Results showed linear relationships, low limits of LOD and LOQ with good robustness.

GRAPHICAL ABSTRACT



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ABSTRACT

A spectrofluorometric method has been developed and validated for the determination of gemfibrozil. The method is based on the excitation and emission capacities of gemfibrozil with excitation and emission wavelengths of 276 and 304 nm respectively. This method allows de determination of the drug in a self-nanoemulsifying drug delivery system (SNEDDS) for improve its intestinal absorption. Results obtained showed linear relationships with good correlation coefficients ($r^2 > 0.999$) and low limits of detection and quantification (LOD of 0.075 μg mL^{-1} and LOQ of 0.226 μg mL^{-1}) in the range of 0.2–5 μg mL^{-1}, equally this method showed a good robustness and stability. Thus the amounts of gemfibrozil released from SNEDDS contained in gastro resistant hard gelatine capsules were analysed, and release studies could be performed satisfactorily.

Introduction

Gemfibrozil (Gem), 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, is a benzene derivative of valeric acid (chemical structure provided in the supplementary data) belonging to a drug group known as fibrates. It has been the clinical choice for the hyperlipidemia (type III) and hypertriglyceridemia (type IV) [1] and it has been found to decrease serum triglycerides and very low density lipoprotein–cholesterol and to increase high density lipoprotein–cholesterol [2] by activating the peroxisome proliferator activated receptors (PPARs), acting mainly on the PPARα isoform [3,4].

Due to its physicochemical properties Gem is a small molecule with poor water solubility, around 0.01 mg mL^{-1} [5] and low dissolution rate in the gastrointestinal tract, which limits its effective absorption and bioavailability after oral administration. Thus, it

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can be assumed that the low oral bioavailability of Gem is due to its solubility and dissolution limitations [5]. Currently, Gem pharmaceutical forms found in the market are tablets and capsules.

In our previous study [6], a self-nanoemulsifying drug delivery system (SNEDDS) was used to conquer the challenge of improving the rate and extent of absorption of Gem, in which the active was loaded in the oily phase. These Gem SNEDDS exhibited a lot of difficulties to be analysed through the most reported technique, high performance liquid chromatography (HPLC) with ultraviolet detection at 276 nm [7,8] due to the excipients included in the formulation. SNEDDS are isotropic mixtures of drug, lipids and surfactants, usually with one or more hydrophilic co-solvents or co-emulsifiers that form fine oil in water nanoemulsions upon mild agitation in an aqueous medium with a droplet sizes ranging 20–200 nm [9]. Different bulk proportions of the mixture components and their physical properties (such as particle size) and the use of different ingredients (such as different excipients) produce changes in the signals from the active ingredients [10]. Spectrofluorometry has long been applied in the field of pharmaceutical analysis of many drugs, being necessary condition for a compound to fluoresce that it absorbs light in the UV or visible region of the spectrum. Most of the additives or excipients found in pharmaceutical preparations are not fluorescent in nature, but compounds that have a conjugated π-electron system may give efficient reemission of the absorbed energy. In this context, the fluorescence characteristics of Gem let the use of spectrofluorometry as detection technique. This is a technique based on the measurement of the relative fluorescent intensity and the wavelength distribution of fluorescent light emitted from an excited molecule through the absorption of radiant energy [11] providing an attractive methodology because of sensitivity, speed, and simplicity, having a low cost and allows the determination of Gem with enough reliability.

The United States Pharmacopoeia (USP) [12], and the International Conference on Harmonization (ICH) [13] provide a clear description of analytical procedures which govern the validation parameters, that need to be evaluated. These parameters are: accuracy, precision (repeatability, intermediate precision), specificity, detection limit, quantification limit, linearity and range.

Therefore, the objective of this work was to develop an accurate, specific and reproducible spectrofluorometric method for the determination of Gem in SNEDDS pharmaceutical dosage forms under study with good sensitivity, relatively simple sample preparation and short run times.

Materials and methods

Reagents

Gemfibrozil of analytical reagent grade was purchased from Sigma–Aldrich (Madrid, Spain), methanol and glacial acetic acid (both of HPLC grade) were purchased from Panreac Química S.A.U. (Barcelona, Spain). For the SNEDDS elaboration: Gem was kindly provided by Menarini Lab., S.A. (Badalona, Spain); oils: Lemon essential oil, anise essential oil, peppermint essential oil, soybean oil, as well as, polyoxy 35 castor oil (Cremophor® EL), were purchased from Fagron Iberica S.A.U. (Terrassa, Spain). Mono/diglycerides of caprylic acid (Capmul® MCM–C8) was kindly supplied by Abitec Corp. (Jamesville, USA). Lauroyl macrogol-6 glycerides EP/lauroyl polyoxyl-6 glycerides NF (Labrafil® M2130CS) from Gattefossé (Saint-Priest Cedex, France). Potassium dihydrogen phosphate, sodium hydroxide 0.2 M, used to prepare buffer solution were purchased from Panreac Química S.A.U. (Barcelona, Spain). Ultra pure water used to prepare all the aqueous solutions was obtained from a Milli-Q® Gradinet A10 system apparatus (Millipore Iberica S.A.U., Madrid, Spain).

Apparatus

Fluorescence spectra and measurements were made on a SFM 25 Kontron spectrofluorimeter (Kontron Instruments, Basel, Switzerland) equipped with a double beam optical system, wavelength range of 200–800 nm, wavelength accuracy of 1.0 nm and wavelength precision of 0.1 nm. For the measurements 1.0 cm quartz cells were utilized and a xenon lamp, with excitation and emission wavelengths of 276 and 304 nm respectively. The analysis was carried out at ambient temperature (23 ± 1 °C). The target was just a mobile phase (solution mixture of methanol of analytical reagent grade and glacial acetic acid of analytical reagent grade 1% v/v) for the calibration curve and a mobile phase plus excipients for the analysis of the samples. The voltage was 320 V. The instrument was calibrated before of this study with good results.

Sample preparation

The Gem loaded SNEDDS formulations were prepared as follows, accurately weighed Gem was mixed with lemon essential oil in a clear screw thread glass vial. Then, the vial was placed into a water bath using open bath circulators MP-5 (Julabo Labortechnik GmbH; Seelbach, Germany) at 40 °C for 20 min with gentle stirring to melt the oily mixture and facilitate the solubilization of Gem. Cremophor® EL and Capmul® MCM-C8 were accurately weighed and added to the oily mixture using a positive displacement pipette. All weighs were measured using PJ 360 Delta Range analytical balance (Mettler-Toledo; Barcelona, Spain). Formulations were stirred gently using a magnetic stir bar and a magnetic stir plate MELB1719 (Merck Eurolab; Lutterworth, UK) until reaching homogeneous solution. The resulting Gem SNEDDS formulations under study contained Lemon essential oil as oily phase, Cremophor® EL as surfactant and Capmul® MCM–C8 as co-surfactant. Loading amount of Gem was 300 mg (F1). Equally, no Gem loaded SNEDDS were elaborated (placebo). The composition of developed formulations is shown in Table 1. The method was based on previous studies of this research group [6].

Preparation of reference standard solutions for the calibration curve

Working standard solutions for the calibration curves were prepared daily as follows; 10 mg of reference standard Gem was accurately weighed and transferred to a 100 mL volumetric flask. Then it was dissolved in a glacial acetic acid 1% (v/v) methanol solution to obtain a final Gem concentration of 100 $\mu g\ mL^{-1}$. From this solution, standard stocks of 5, 4, 2, 1, 0.5 and 0.2 $\mu g\ mL^{-1}$ were prepared and thermostated at 25 ± 0.1 °C in a temperature controlled bath Jubalo MP-5 (Julabo Labortechnik GmbH, Seelbach, Germany).

Analysis of Gem in SNEDDS

A quantity of SNEDDS equivalent to a 100 mg of Gem was accurately weighed by precision balance PJ360 DeltaRange (Mettler Toledo, Barcelona, Spain) and placed in a 100 mL volumetric flask and filled with the mobile phase. The solution was mixed until its complete dissolution. This solution was further diluted to obtain Gem concentration of 2 $\mu g\ mL^{-1}$ and thermostated at 25 ± 0.1 °C in a temperature controlled bath. Then the fluorescent intensity was measured.

The possible interferences from the excipients in the SNEEDS formulations were evaluated. As a control, equal quantity of SNEEDS formulation containing no Gem (placebo) was prepared similarly and fluorescent intensity measured at 304 nm after excitation at 276 nm.