

VALIDATION OF ANALYTICAL METHOD BY UV SPECTROPHOTOMETRIC QUANTIFICATION OF GEMFIBROZIL INCORPORATED IN THE MICROEMULSIONS

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ABSTRACT

The present study aims to validate an analytical method by UV spectrophotometric for the quantification of gemfibrozil incorporated into microemulsions. Experimental development by constructing a ternary phase diagram for the identification of microemulsions. The characterization of the microemulsions was

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carried out by the polarized light microscopy technique. The analytical method was validated according to the parameters described by the United State Pharmacopeia (USP) 39 NF34 and International Conference on Harmonization (ICH) (2005), which evaluated linearity, robustness, intra and inter day accuracy, specificity accuracy, limits of detection and quantification. The developed method demonstrated linearity with a coefficient of determination (R^2) of 0.9898, in addition to precision, accuracy and robustness for each of the tests with values that presented a coefficient of variation of less than 5 %. The results obtained were favorable, since they comply with the parameters stipulated by USP 39-NF34 (2016) and ICH (2005), indicating that the method is effective for the intended purpose.

INTRODUCTION

The prolonged drug release systems are classified as new technological alternatives used to control the concentration and time of drug release after administration. These systems can be solid dispersions, micro and nanoparticles, liposomes, microemulsions etc. (Huo et al., 2017; Davoodi et al., 2018; El Maghraby, Arafa, & Essa, 2018).

Microemulsions (MEs), are systems composed of water and oil that are stabilized by surfactants, their main characteristic is thermodynamic stability, due a large amount of energy is not required for their formation.

Unlike emulsions, microemulsions have transparent aspects, this is due to the small size of the particles, which can reach 500 nm. ME can be characterized as oil in water (O/W) and water in oil (W/O), which confers the particularity of being able to incorporate lipophilic and hydrophilic drugs (Callender et al., 2017; Ferreira et al., 2018; Gvaramia et al., 2018).

Gemfibrozil (GFZ) is a drug that acts as a cholesterol reducer, derived from fabric acid and chemically known as 5- (2,5-dimethylphenoxy) -2,2-dimethylpentanoic acid. Its prescription is

also made in order to reduce the risk of stroke, heart attack or other cardiac complications as it increases high density lipoproteins (LDL) (Tornio et al., 2017). Despite having studied the interactions, it is not yet possible to accurately quantify the drug within the systems matrix due to the lack of validated methods for this purpose.

The validation of a methodology is important as it aims to demonstrate the suitability of the method to carry out an analysis. The methods of analysis used in the quality control of pharmaceutical products must have been validated prior to routine use. The validation of a test method is intended to demonstrate the suitability of the method to carry out an analysis (USP 39 NF 34 2016; ICH, 2005; Cádiz & García, 2015).

Ultraviolet-Visible Spectroscopy (UV-Vis) is a technique that is based on

the ability of certain molecules to absorb radiation such as those corresponding to the visible UV spectrum. The use of a spectrophotometer allows readings in a wavelength range of 190 to 800 nm, simultaneously considering factors such as atomic structure, medium conditions (pH, temperature, ionic strength, dielectric constant) which finally makes the information provided of the energy states, atomic or molecular, of the study sample allow its determination and characterization. The UV-spectrophotometry is an inexpensive, easy, reliable, fast and useful, meets the requirements of quality control of pharmaceutical and cosmetic industries. As an alternative to the existing method by HPLC (Valcárcel, González y Valcárcel, 2008; Cubas et al., 2018; Maldonado et al., 2018). The aim of this work is to validate an analytical method to gemfibrozil incorporated in the microemulsions.

MATERIALS AND METHODS

Materials

Sodium Chloride (NaCl), Potassium Chloride (KCl), Calcium Chloride

(CaCl₂), Sodium Phosphate (NaHPO₄), Glucose (C₆H₁₂O₆), Sodium Hydroxide (NaOH), Gemfibrozil were purchased from Sigma-Aldrich CO (USA).

Methods

Phase diagram construction

The ternary phase diagram was prepared using tween80, oleic acid and Milli Q water at 25 °C. In the mixture of water/oil was added the surfactant until the solution is completely transparent, which was indicative of the formation of the single phase. Once the candidate sample was separated, the sample was prepared with and without drug for the validation of the analytical methodology.

Polarized light microscopy

The microemulsion structure was analyzed by means of a photomicroscope (Leica DMLP microscope) equipped with a camera: firstly, under the bright field and secondly, under the polarized light (using cross-polarizers) in order 5 to investigate the presence of the liquid crystalline phase in each sample. Leica IM 1000 software was used to analyze the obtained micrographs.

Preparation of buffer TC199 with glucose at pH 7.4

The buffer was prepared according described by Silva et al (2008). Briefly

145 mM NaCl; 4.56 mM KCl; 1.25 mM CaCl₂ 2H₂O; 5 mM NaHPO₄ were weighed and leading to a final volume of 1000 mL of Milli Q water, the glucose was added, and the solution was adjusted to a pH of 7.4 using 1N NaOH.

Analytical method validation

The methodology was performed using the parameters defined by USP 39 NF 34 (2016) and ICH Q2 (2005). All analyzes were performed at a wavelength of 276 nm, this value refers to the maximum absorption wavelength of GFZ (USP 38, 2015).

Linearity

The linearity of the method was determined by obtaining three analytical curve of gemfibrozil/microemulsion (GFZ/MEs) in absolute ethanol (250 mg/mL). Transferred aliquots of stock solution to a volumetric flask to obtain nine concentrations, from 0.02 to 0.09 mg/mL of GFZ and the volume was completed with absolute ethanol, then the absorbance-absorbance of the samples were measured in 276 nm using blank ethanol. The dilutions were prepared in triplicate.

Precision

The data were analyzed at a wavelength 276 nm. The precision of the analytical curves was determined in three consecutive days for intra- and inter-day.

Accuracy

The accuracy is the closeness of the results obtained by the method under study in relation to the true value for the quantitative analysis (ICH 2005). The accuracy was determined by testing the recovery of GFZ in ethanol, which was calculate the percentage of recovery of known amount of drug added to the sample using Equation 1.

$$\% \text{Recovery} = Y/X \times 100 \% \quad (1)$$

Y = absorbance of the sample; X = standard absorbance; acceptance criteria for recovery: 98-102 %.

Limit of detection (LOD), limit of quantitation (LOQ)

To determine the LOD and LOQ, three solutions prepared in triplicate, near the lower limit of the analytical curve. The LOD and LOQ were cal-

culated based on the equations (2 and 3) as describe in ICH (2005), it is expressed as:

$$\text{LOD} = 3(\text{S.D./}a) \quad (2)$$

$$\text{LOQ} = 10(\text{S.D./}a) \quad (3)$$

where S.D of y-intercepts and S is the slope of the calibration curve.

Robustness

The robustness should show the reliability of an analysis related to deliberate variations in method parameters ICH (2005). The robustness was determined by analyzing the sample under the variation of the pH and manufacture of ethanol.

Specificity

The specificity was carried out under two standards: gemfibrozil and microemulsion without the drug, to determine that there would be no interference from the excipients present in the formulation.

Encapsulation efficiency

The entrapment efficiency (EE) was

determined to access the extent of GFZ incorporation in the microemulsion. EE of GFZ-loaded MEs was determined by measuring the concentration of the free unloaded GFZ in the aqueous medium of the MEs suspension. Briefly, one gram of GFZ-loaded

MEs was weighted and ultracentrifuged for 20 min at 15000 rpm, approximately. Then, the amount of GFZ in the supernatant was determined by UV spectrophotometric analysis. The entrapment efficiency (EE) was calculated by the equation 4.

$$EE (\%) = \frac{\text{Total mass of GFZ} - \text{mass of GFZ in aqueous phase}}{\text{Total mass of GFZ}} \times 100 \quad (4)$$

RESULTS

Phase diagram construction

In the present study a ternary phase diagram was constructed and microemulsion region was found using surfactant concentration rate between 10-40 %. The Figure 1 exhibit the phase diagram and the photograph of the selected microemulsion.

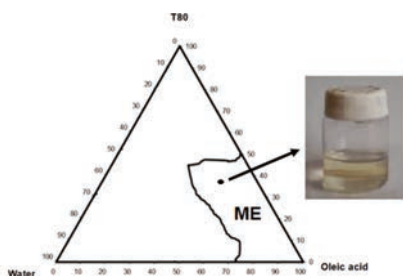


Figure 1. The photograph of selected microemulsion

Polarized light microscopy

The Figure 2 exhibit eh light microscopy to microemulsion containing GFZ

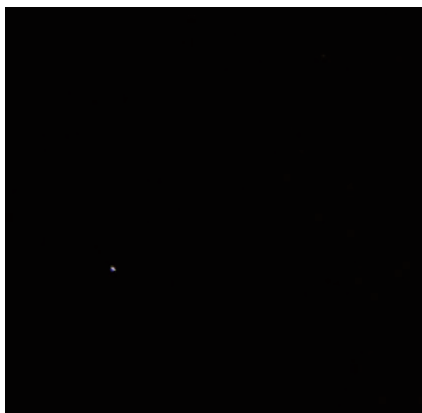


Figure 2. Light microscopy to microemulsion containing GFZ

Validation of analytical method

Linearity

In order to obtain the linearity results, a calibrated analytical curve is cons-

tructed, so that the dilutions are elaborated from a parent solution, the value obtained from R^2 is 0.9984 as shown in Figure 3 and the standard deviation values.

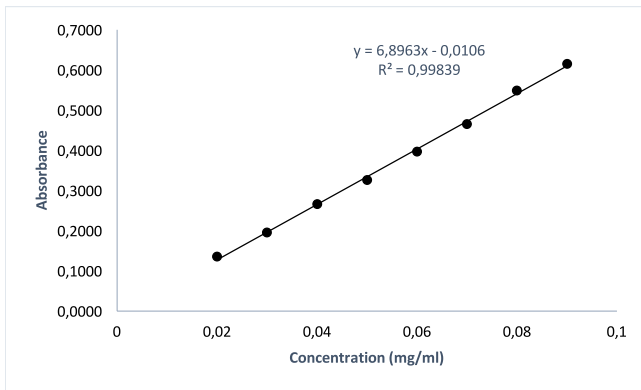


Figure 3. The linearity results

The standard deviation (SD) and coefficient of variation (CV) data is shown in Table 1.

Precision

This parameter was assessed by applying the intra and inter day tests through the analysis of 3 samples of different concentrations, the minimum (0.02 mg/mL), the medium (0.05 mg/mL) and the maximum (0.09 mg/mL) as showed in the Table 2.

Table 1. Standard deviation and coefficient of variation of the curve data

Concentration (mg/mL)	Average	DS (±)	CV %
0.09	0.6152	0.0102	1.66
0.08	0.5492	0.0100	1.82
0.07	0.4651	0.0027	0.58
0.06	0.3964	0.0028	0.72
0.05	0.3265	0.0042	1.29
0.04	0.2662	0.0045	1.69
0.03	0.1950	0.0035	1.79
0.02	0.1358	0.0022	1.61

Table 2. Standard deviation and coefficient of variation of the Inter and Intraday precision

Interday				Intraday			
Concentration (mg/mL)	Average	SD (±)	CV (%)	Concentration (mg/mL)	Average	SD (±)	CV (%)
0.09	0.5963	0.0029	0.48	0.09	0.6009	0.0006	0.10
0.05	0.3262	0.0004	0.13	0.05	0.3263	0.0002	0.07
0.02	0.1346	0.0004	0.27	0.02	0.1358	0.0005	0.37

Accuracy

The accuracy of the proposed method was assessed by determining the

average recoveries of samples using the standard addition method. The

Table 3 show the results of accuracy.

Table 3. Accuracy test results

Concentration (mg/mL)	Average	SD (±)	CV %	Recovery (%)
0.09	0.5968	0.0018	0.30	101.15%
0.05	0.3389	0.0014	0.41	101.16%
0.02	0.1350	0.0005	0.39	99.11%

Specificity

The specificity of the method was evaluated under 2 standards gemfibrozil and microemulsion without

drug, in order to determine that there was no interference from the excipients present in the formulation as shown in Figure 4.

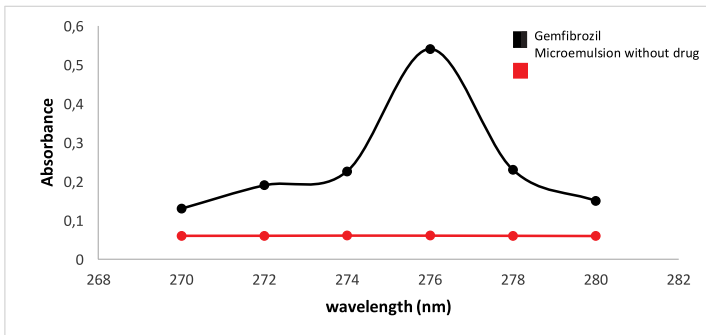


Figure 4. Specificity of microemulsion without gemfibrozil

Robustness

The robustness test was realized modifying the manufacture and the pH of the buffer solution. These param-

eters were selected due the ease access. The results of the selected variables in the robustness assessment are shown in Table 4.

Table 4. Robustness test results

Concentration 0.05mg/mL	Buffer pH 7.0			Buffer pH 7.4		
	Average	SD (±)	CV %	Average	SD (±)	CV %
Manufacture 1	0.3267	0.0001	0.018	0.3263	0.0003	0.08
	0.3264	0.0002	0.047	0.3264	0.0002	0.047
	0.3264	0.0002	0.071	0.3263	0.0002	0.064
Manufacture 2	0.3262	0.0001	0.018	0.3265	0.0001	0.018
	0.3260	0.0002	0.047	0.3263	0.0002	0.064
	0.3259	0.0002	0.0469	0.3264	0.0001	0.0177

LOD and LOQ

The detection limit is the lowest amount of the analyte that can be detected but do not necessarily quantified, while the limit of quantification is the lowest amount of the analyte that can be determined with accuracy and precision ICH (2005). The LOD and LOQ values were 0.062 and 0.189 mg/mL respectively, this results indicate the excellent sensitivity of the analytical method.

Encapsulation efficiency

The encapsulation efficiency to GFZ was 88 % at the MEs. The results for both NLCs analyzed are close to those described by Addas et al. (2019) and were satisfactory, since they show high entrapment efficiency in the matrix of MEs.

DISCUSSION

In the present work, we have validated a method to quantification of gemfibrozil incorporated in the microemulsions. It is possible to observe a transparent solution with a

single phase which indicates the formation of microemulsion. It is suggested due the spontaneous formation and thermodynamic stability, and a dark field was observed in the

polarized microscopy which suggest the microemulsion formation. It was possible to observe the presence of a black field, which can suggest microemulsions formation. According to Froelich et al. (2017), a main feature of these microemulsion systems is dark field observation.

To validation method, it was demonstrates the linearity expressed by the relationship between the concentration of gemfibrozil present obtaining a value of R^2 0.9984, indicating that the curve is linear for the intended purpose. Similar results were found by Gaete (2014), the author validated an analytical methodology for the application of paracetamol in a bioexemption study, and obtained a value of 0.999 in the R^2 , which indicated that the curve is linear for the purpose of the work.

The acceptance of linearity will depend on the value obtained in the correlation coefficient and the intersection of the line are its axis. This value indicates the linear regression of the concentration against absorbance. The correlation coefficient value equal to or greater than 0.99 is normally acceptable, since it indicates

that the analytical curve is linear USP 39 NF 34 (2016). In this context, the analytical curve is considered as a reference for the quantification of gemfibrozil.

The data presented in Table 2 showed that the method is precise for the quantification of gemfibrozil incorporated at the microemulsion. The coefficient of variation values was below 0.5 %, which indicate that the method is sufficiently precise. The results are in accordance with the provisions of the ICH (2005), which suggests that the coefficient of variation for this parameter should be less than 3.0 %. Cáceres, et al (2016) obtained a coefficient of variation of less than 1 %, with the results presented, both authors argue that the method is accurate. With the above it is shown that the method is accurate for the intended purpose.

The results presented in the Table 3 are in accordance with the official validation regulations. The ICH (2005) stipulates that the recovery percentage must be in a range of 80 to 120 %. Lozano et al., (2018) validated an analytical method for the quantification of alprazolam in ta-

blets, in the precise parameter, the results obtained a recovery of 100.69 %, in this way, the authors indicated that the parameter is within the limits stipulated by ICH (2005) and USP 39 NF34 (2016).

The specificity of the drug could be determined by a wavelength scan, where we can show that the highest

reading peak corresponds to the 276 nm suggested by USP 39 NF34 (2016) as the wavelength for GFZ. In addition, it can be evidenced that no interference is generated between ME without drug and the sample with GFZ demonstrating that microemulsion excipients do not interfere with gemfibrozil reading.

CONCLUSION

In the present project, a method proposed an analytical derivative UV spectrophotometric was developed. This method showed advantages related to the simplicity, fastness and low-cost conditions. All validation parameters were satisfactory, the re-

sults showed that the method is suitable for quantification of gemfibrozil incorporated in microemulsion, as consistent with the requirements of ICH, thus providing reliability to the analyses proposed.

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