DOI: 10.21276/sajp.2016.5.8.7

Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2016; 5(8): 350-358 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Original Research Article

Development of Analytical Method Applied to Preformulation Studies of a Novel Benzophenone Derivative with Pharmaceutical Interest

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Abstract: In this work, a selective and sensible analytical method by reversed phase high performance liquid chromatography was developed and validated. The method was applied in several preformulation assays of a novel benzophenone derivative denominated LFQM117. This compound was selected due to its potential as leishmanicidal drug candidate to topical therapy. Preformulation assays included the LFQM117 physicochemical characterisation as solubility in different vehicles, oil-water partition coefficient (*log P*) and *in vitro* skin absorption studies of LFQM117. Forced stress tests were also performed to assess the intrinsic stability of this compound. The results showed that the analytical method developed was suitable to be employed in the preformulation tests carried out in this work. The preformulation studies indicated that LFQM117 showed low water solubility and high lipophilicity (*log P* = 4.8). And the LFQM117 remained stable under forced stress conditions. The skin absorption studies showed that LFQM117 permeation through the porcine ear skin was significantly increased by addition of oleic acid (absorption enhancer). And this study was the starting point for the development of topical formulations with LFQM117.
Keywords: Benzophenone derivatives, Analytical method. Preformulation, Stability, Skin absorption, Topical therapy.

INTRODUCTION

Benzophenone derivatives (BPDs) are aromatic compounds formed by two aromatic rings linked by a carbonyl moiety with different substituents in the benzene moieties that significantly alter their stereo electronic properties. These natural or synthetic BPDs have been studied due to their different physicochemical properties and biological activities, such as photo protection [1], antimicrobial [2,3], anti-proliferative [4], anti-inflammatory [5-7] and leishmanicidal [8,9].

Recently, a synthetic BPD (Figure 1) previously denominated LFQM117 (2-hydroxy-4-*O*-(3,3-dimethyl)-allylbenzophenone) showed leishmanicidal activity [9,10] and it was selected as a drug candidate for topical administration to be used in different types of diseases such as Leishmaniosis and skin disorders.

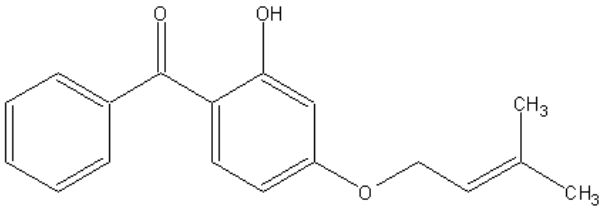

Fig-1: LFQM117 chemical structure.

The incorporation of selected drug candidates in topical formulations demands previous characterisation of the molecule, including their physicochemical properties and percutaneous absorption (penetration and permeation). Thus, preformulation studies must be applied to physicochemical characterisation of the drug, ensuring the quality and efficacy of the developed formulations. The knowledge of physicochemical properties of the compound such as solubility in different vehicles, oil-water partition coefficient (*log P*), and intrinsic stability must be previously determined during the topical formulations design.

An important step is to develop selective analytical methods to drug analysis during formulations development e.g. using chromatographic techniques [11-17]. For topical formulations, the percutaneous absorption of the compound is a key parameter to be determined in order to achieve the success of the topical therapy considering the barrier function of the skin. And a sensitive and selective analytical method becomes indispensable.

The aim of this work was to develop and validate a selective and sensible analytical method to be applied in preformulation studies of the drug candidate LFQM117, for the development of topical formulations. The preformulation assays included the LFQM117 physicochemical characterisation as solubility in different vehicles and oil-water partition coefficient (*log P*). Stress testing, thermo gravimetric analyses using differential thermal analysis (TGA-DTA) and studies by attenuating total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) with a hot stage apparatus were employed to assess the stability of LFQM117. And the skin absorption of LFQM117 was evaluated using porcine ear skin and vertical diffusion cell.

MATERIAL AND METHODS

Chemical and reagents

All reagents were of analytical grade. Acetic acid and methanol for high performance liquid chromatography (HPLC) were purchased from Vetec® (Rio de Janeiro, Brazil). Chloride acid, ethanol, hydrogen peroxide (H_2O_2), isopropyl alcohol, n-octanol, potassium chloride, potassium hydrogen phosphate, sodium chloride (HCl), sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium hydroxide (NaOH), sodium dodecyl sulphate (SDS), Tween 20, and Tween 80 were purchased from Vetec® (Rio de Janeiro, Brazil) as well. Cremophor EL and Poloxamer 407 were acquired from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade water was prepared by Milli-Q reverse osmosis (Millipore, Billerica, MA, USA). 2-hydroxy-4-*O*-(3,3-dimethyl)-allylbenzophenone (LFQM117) was synthesized and purified by Phytochemical and Medicinal Chemistry Laboratory of UNIFAL-MG according to previously report [10].

Instrumental

Samples were analysed by a LC-10A HPLC series from Shimadzu (Kyoto, Japan) with a SPD-M10AVP diode array detector. Vertical diffusion cell was from Hanson Research Corporation (Chatsworth, CA, USA) with effective diffusion area of 1.77 cm^2 and volume of 7 mL. ATR-FTIR model Nicolet IS50 (model GladiATR 300, Pike Technologies, USA) with hot stage plate (model iS50, Thermo Scientific, USA). TGA-DTA model TG/DTA 7300 was from Exstar (SII Nano Technology, Japan). Hot plate magnetic stirrer model MA-085 was from Marconi (Brazil). Analytical balance model AUY220 was from Shimadzu (Kyoto, Japan).

RP-HPLC analysis

The LFQM117 HPLC analysis was performed using a Merck (Germany) LiChrospher® RP-select B column (125.0 x 4.0 i.d.) mm with particles of 5 µm and Merck pre-column LiChrospher® RP-select B column (4.0 x 3.0 i.d.) mm. The mobile phase was a mixture of 0.1% acetic acid and methanol (80:20). The mobile phase flow rate was 1.0 mL min^{-1}, the injection volume was 50 µL, and ultraviolet (UV) detection was carried out at 290 nm. The UV spectrum in the range of 190-370 nm was investigated to select the wavelength to drug analysis. The samples were filtered using a 0.45-µm syringe filter composed of hydrophilic poly tetra fluoro ethylene (PTFE). The retention time of LFQM117 was approximately 4.7 min. The run time was 15 min.

Analytical method validation

The method validation was carried out following the Food and Drug Administration recommendations published in the Guidance for Industry: Bio analytical Method Validation [18]. Samples were prepared in a mixture of methanol and water (80:20).

Selectivity

Selectivity assay was evaluated using standard solutions and samples from *in vitro* percutaneous absorption studies (permeation and penetration). All solutions in theoretical concentration of 10.0 µg mL^{-1} were injected into the chromatographic system. Diluents and blank solutions were analysed in the absence of LFQM117 to assess possible interferences in the retention time of this compound.

Linearity

Linearity was evaluated to demonstrate the proportionality relationship between the concentration of the standard solutions of LFQM117 and the detector response (peak area). For this, a standard stock solution of LFQM117 in methanol (1.0 mg mL^{-1}) was prepared and successive dilutions were performed to obtain standard solutions at concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5, 10, and 20 µg mL^{-1}. The assay was performed in three replicates and the correlation coefficient (*r*) and the equation line (y = ax + b) were calculated, which *a* corresponds to the slope and *b* is the linear coefficient (intercept).

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were determined from the slope and the standard deviation of the y-intercept from the three replicates of the linearity assay according to the following equations:

$$LOD = \frac{SD\ x\ 3{,}3}{IC} \quad (1a)$$

$$LOQ = \frac{SD\ x\ 10}{IC} \quad (1b)$$

Where *SD* is the standard deviation of the y-intercept of regression line, and *IC* is the inclination (slope).

Accuracy and precision

Accuracy and precision were established from six replicates of LFQM117 standard solutions at three concentration levels (0.1, 1.0, and 10.0 µg mL^{-1}). Analyses were performed on three consecutive days.

The intraday and interday precisions were established as the dispersion of the measurements around the average value and expressed mathematically by the relative standard deviation (RSD), according to the equation 2:

$$RSD = \frac{Standard\ value}{Mean\ value}\ x\ 100 \qquad (2)$$

The intraday and interday accuracy were presented as relative errors (*RE*) and were calculated by equation 3, as follows:

$$\mathrm{RE} = \frac{\text{Experimental concentration} - \text{Theoretical concentration}}{\text{Theoretical concentration}} \text{ x } 100 \qquad (3)$$

Drug recovery from skin samples

LFQM117 was recovered from porcine ear skin spiked with known amounts of the compound. Aliquots (10.0, 20.0 e 30.0 µL) of standard solution (2.0 mg mL^{-1}) were applied to excised skin (1.77 cm^2). Three concentration levels (11.3, 22.6 and 33.9 µg cm^2) were spiked in skin samples. These studies were performed in triplicate. Skin samples were placed in polyethylene tubes and allowed to stand for 1 hour to ensure LFQM117 penetration. The LFQM117 extraction was performed using 5 mL of methanol. Skin homogenates were prepared by triturating skin fragments using a TURRAX homogenizer (Brazil) model MA102. The supernatant was filtered through quantitative filter paper (J Prolab, Germany) followed by filtration using 0.45-µm syringe PTFE filter before the injection in the HPLC system. LFQM117 recovery (R) was calculated according to the equation 4.

$$\mathrm{R} = \frac{\text{Experimental concentration}}{\text{Theoretical concentration}} \text{ x } 100 \qquad (4)$$

Filter effect

The filter effect study was performed by measurements of standard solutions (0.1, 1.0 and 10.0 µg ml^{-1}) before and after the filtration process. These studies were performed in triplicate.

Preformulation

Solubility

LFQM117 solubility in different vehicles was determined by the shaking flask method [19]. The vehicles evaluated were water, ethanol, isopropyl alcohol, phosphate buffer pH 5 and phosphate buffer pH 7.4. The effect of surfactants at 5% m/v was evaluated in phosphate buffer pH 7.4: Poloxamer 407, Sodium dodecyl sulphate, Tween 20, and Tween 80. The non-ionic surfactant Cremophor EL at 10% m/v was also evaluated in phosphate buffer pH 7.4. In these evaluations, an excess of LFQM117 was added in 10 mL of each vehicle and stirred at 300 rpm for 24 h at 25°C. Samples were filtered through quantitative filter paper (J Prolab, Germany) followed by filtration using 0.45-µm syringe PTFE filter before the injection in the HPLC system.

Log P

The *log P* was determined according to method previously reported using HPLC [20, 21]. For this, a solution of LFQM117 100.0 µg mL^{-1} in methanol was injected (20 µL) in Waters (Ireland) XTerra RP C_{18} column with particles of 5 µm (100x4.6 mm i.d). The injections were performed in triplicate and UV detection was carried out at 254 nm. Analyses were performed in five different mixtures of mobile phases (methanol with 0.25% v/v of n-octanol and phosphate buffer 10 mM pH 7.4) at 0.5 mL min^{-1}. The retention factor (k') was calculated in each mobile phase condition. These results were employed to build a graph log k' versus decimal of organic solvent percentage. After linear regression the value of k'_w was determined as the extrapolation to 100% of aqueous solvent. The *log P* was calculated by the equation *log P* = 0.13418+0.98452 log *k'w*. Results obtained experimentally were compared with theoretical values calculated by ACD/I-Lab software (Version 6.0 for Microsoft Windows).

Stability

The LFQM117 stability was evaluated across forced degradation studies and other methods such as thermal analyses (TGA-DTA) and ATR-FTIR with hot stage.

Forced degradation studies

LFQM117 forced degradation was evaluated in different conditions. LFQM117 solutions, in the theoretical concentration of 20 µg mL^{-1}, were subjected to thermal stress (60°C) in ultrapure water for 24 hours to give a high degradation rate. LFQM117 solutions in the same theoretical concentration were also subjected to acid stress (0.01 mol L^{-1} HCl and 0.1 mol L^{-1} HCl), alkaline stress (0.01 mol L^{-1} NaOH and 0.1 mol L^{-1} NaOH), oxidative stress (3% H_2O_2 and 10% H_2O_2) and direct light exposure. After 24 h, the samples were analysed by HPLC in order to separate and detect possible degradation. The recovery was determined in relationship to the theoretical value defined as 100%, according to equation 4.

LFQM117 standard solution (1.0 mg mL^{-1}) was also subjected to low temperature (4°C) for 24 hours to detect possible compound precipitation.

ATR-FTIR with hot stage

The spectra were recorded from 30 to 180 °C at a heat rate of 10 °C.min^{-1} and collected one co-added spectrum of 32 scans per minute. LFQM117 was applied directly to the crystal and the spectra were obtained in the wavelength range of 3500-500 cm^{-1} using a resolution of 4 cm^{-1}.

TGA-DTA

TGA-DTA measurements were performed under a dynamic atmosphere of nitrogen (50 mL min^{-1}) with a heat flow of 10 °C min^{-1} from 30 to 500 °C using

open aluminum pans. The equipment was calibrated using an indium standard for temperature and an alumina calibration weight for mass.

In vitro skin absorption studies

The *in vitro* skin absorption studies (penetration + permeation) were performed using excised porcine ear skin. Whole skin was excised after animal´s slaughter Olhos d´Agua slaughterhouse (Ipuã-SP-Brazil). Nerves, blood vessels, hairs, and the adipose tissue layer were removed with the aid of a surgical scalpel and scissors. Excised porcine ear skin was frozen and stored at -70 ºC (Glacier Ultralow Temperature Freezer) for up to three months. Porcine ear skin (700 µm) was prepared before the skin absorption studies using a dermatome (model 1990 Nouvag AG, Swiss). Excised skin samples without superficial damage were placed on the donor chamber of the vertical diffusion cell. The receptor chamber was previously filled with phosphate buffer pH 7.4 + 10% Cremophor EL. The Cremophor EL was added to receptor medium to guarantee the sink conditions. The receptor medium was stirred at a constant speed of 600 rpm, and temperature was adjusted to 32.0 ± 0.1 ºC using a circulating water bath.

LFQM117 dispersions (1% m/v) were prepared in mineral oil with or without oleic acid (5 % m/v). 500 µL of LFQM117 dispersion were applied to the skin surface (stratum corneum), and samples (2 mL) were withdrawn after 3, 6, and 9 h and were immediately replaced with an equal volume of receptor phase. Samples were analysed by RP-HPLC method to evaluate LFQM117 permeated. After 9 h, skin was removed from diffusion cell in order to analyse the amount of LFQM117 retained in the skin (penetration). Excess of LFQM117 dispersion was removed and skin homogenates were prepared by triturating skin fragments using a TURRAX homogenizer and 5 mL of methanol. The supernatant was filtered through quantitative filter paper followed by filtration using 0.45-µm syringe PTFE filter before the injection in the HPLC system.

RESULTS AND DISCUSSION

The administration of synthetic BPDs has been related to significant systemic toxicity [22, 23, 24]. In order to avoid the systemic effects, the novel synthetic BPD denominated LFQM117 was studied in this work as a potential drug candidate to be incorporated in topical formulations for treatment of several cutaneous disorders. However, the development of topical formulations is not a simple task. For this, preformulation studies are an auxiliary tool to study the incorporation of the bioactive compound in an appropriate pharmaceutical vehicle and to assess the percutaneous absorption of this molecule.

Preformulation studies demand the drug analysis by selective methods, and their physicochemical characterisation including solubility, lipophilicity, intrinsic stability, melting point, and permeability (e.g. through the skin). For analytical determination of bioactive compounds, chromatographic methods are usually required, allowing the drug quantification in different matrices [11-17]. Therefore, we firstly developed a rapid, sensible and selective method by RP-HPLC to LFQM117 determination and then we evaluated its application in the several steps of the preformulation study.

Analytical method

LFQM117 is a non-ionic compound, but it has a slight acid character. So, in the development of the analytical method, an acid mobile phase was selected because it reduces the lipophilicity of the BPD compound and consequently its retention factor. In spite of this, an appropriate efficiency of separation (Theoretical plates > 2500) must be assured. The selected mobile phase to conduct the validation studies was the one employing 0.1% v/v acetic acid: methanol (20:80). Conditions evaluated and system suitability parameters of the liquid chromatographic method development are shown in Table 1.

Table-1: Method development by RP-HPLC

Mobile phase proportions (0.1% v/v acetic acid : methanol)	Retention time (min)	Asymmetry factor	Retention factor (k')	Theoretical plates number (N)
15: 85	3.01	1.19	2.01	2018
20: 80	4.64	1.09	3.64	2591
25: 75	7.81	1.02	6.81	3352
30: 70	14.62	0.98	13.62	4273

Validation studies were carried out in order to demonstrate that the method was appropriate for preformulation studies. Validation parameters evaluated were selectivity, linearity, accuracy and precision, limit of quantification (LOQ), limit of detection (LOD) and LFQM117 recovery from porcine ear skin.

Selectivity studies did not indicate the detection of any peaks at the same time of LFQM117 in the diluents or blank sample (Figure 2). These results demonstrated the selectivity of the analytical method,

indicating that the results were not altered by contaminants from ear porcine skin, vehicles or buffers.

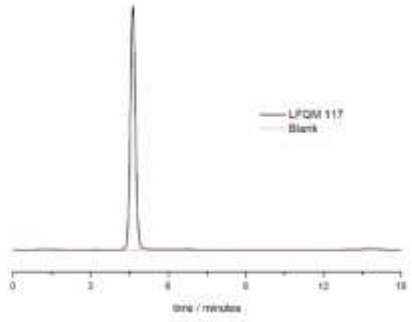

Fig-2: Chromatogram of LFQM117 standard solution and blank solution.

Linearity was assured in the range from 0.10 to 20.00 µg mL^{-1}. The equation line was y = 151686.52 (± 888.63) x + 2572.61 (± 351.43), and the correlation coefficient (r) was 0.999965 (± 0.000007). The values for LOD and LOQ were respectively 7.65 ng mL^{-1} and 23.17 ng mL^{-1}. Accuracy and precision were lower than 15% and were considered satisfactory [18] (Table 2).

Table-2: Accuracy and precision evaluation.

Validation parameters	LFQM117		
Intra assay precision			
Theoretical concentration (µg mL^{-1})	0.1	1.0	10.0
Experimental concentration (µg mL^{-1})	0.102 ± 0.003	1.009 ± 0.001	9.943 ± 0.056
n	6	6	6
RSD (%)	2.94	0.10	0.56
Inter assay precision			
Theoretical concentration (µg mL^{-1})	0.1	1	10
Experimental concentration (µg mL^{-1})	0.098 ± 0.008	1.073 ± 0.057	10.677 ±0.641
n	3	3	3
RSD (%)	8.16	5.31	6.00
Accuracy			
Intra assay (%)	2.00	0.90	- 0.57
Inter assay (%)	-2.00	7.30	6.77

N, determinations; RSD, relative standard deviation

The LFQM117 recovery studies from spiked skin samples indicated that this method was appropriated to evaluate the compound retention in porcine ear skin. The recovery percentages at three concentration levels (11.3, 22.6 and 33.9 µg cm^2) were respectively 97.8 ± 7.8%, 108.8 ± 1.6% and 95.7 ± 4.2%.

Preformulation studies

The preformulation assays included the physicochemical characterisation of the LFQM117 as intrinsic stability, solubility in different vehicles, oil-water partition coefficient (*log P*) and *in vitro* skin absorption studies.

LFQM117 remained stable after forced degradation studies. These evaluations are important to determinate if drug can be degraded during the storage or in the steps during the design of the formulation. Hydrolysis, oxidation, high temperatures, and humidity are possible reasons of drug degradation. The drug recovery in all stress conditions indicated a variation of less than 5% in relationship to the initial amount (Table 3).

LFQM117 solution (1.0 mg mL^{-1}) also remained stable when subjected to low temperature (4°C), since it was not observed crystal growth.

Table-3: LFQM117rRecovery percentage after forced degradation studies

Stress condition	Peak area ± SD	Recovery (%)
0.1 mol L^{-1} HCl	3228000 ± 7000	103.10
0.01 mol L^{-1} HCl	3132000 ± 3000	100.05
0.1 mol L^{-1} NaOH	3260000 ± 90	104.13
0.01 mol L^{-1} NaOH	3122400 ± 1000	99.73
3% H_2O_2	3116000 ± 23000	99.53
10% H_2O_2	3142000 ± 9000	100.35
High temperature (60 ºC)	3160000 ± 30	100.93
Direct light exposure	3100000 ± 2000	99.04

SD, standard deviation

ATR-FTIR studies were applied to identify functional moieties of LFQM117 (Figure 3a). C-H sp^3 symmetric and asymmetric stretching was observed at 2872 and 2963 cm^{-1}. C-H sp^2 symmetric and asymmetric stretching was observed at 2855 and 2924 cm^{-1}. The asymmetric deformation of C-H was observed at 1375 cm^{-1}. C=O stretching was observed at low frequencies (1624 cm^{-1}) due to the conjugation effect with the C=C from aromatic rings. Aromatic C=C was observed at 1443 cm^{-1}. The out-of-plane stretching of benzenes were observed at 789, 752, and 700 cm^{-1}, and C-O stretching was observed at 1107 cm^{-1}. Thermal decomposition of LFQM117 was evaluated by ATR-FTIR with a hot stage plate (Figure 3b).

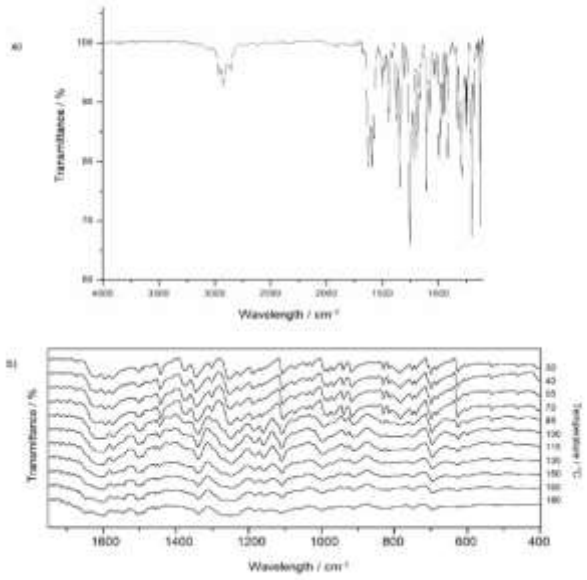

Fig-3: ATR-FTIR spectra of the LFQM117 drug candidate obtained (a) at room temperature and (b) with a hot stage plate.

According to these results, LFQM117 spectrum showed the bands disappearance of LFQM117 at 85ºC, as C=C and C=C stretching. The disappearance, appearance or dislocation of infrared bands was related to alterations of the chemical structure of the analysed compound. According to the DTA curve (Figure 4), the temperature of 85ºC (first thermal event) corresponds to the melting point of LFQM117. TGA-DTA allows characterising the thermal events of several compounds, as melting and boiling points as well to the thermal stability. The mass losses indicated by TGA started at 85°C with a low degradation rate. The mass losses were accelerated at around 200°C, probably due to a major degradation rate of LFQM117 at high temperatures. Thermal decomposition was related to the second event in the DTA curve, supporting this result and at 300°C, mass losses of LFQM117 were practically total.

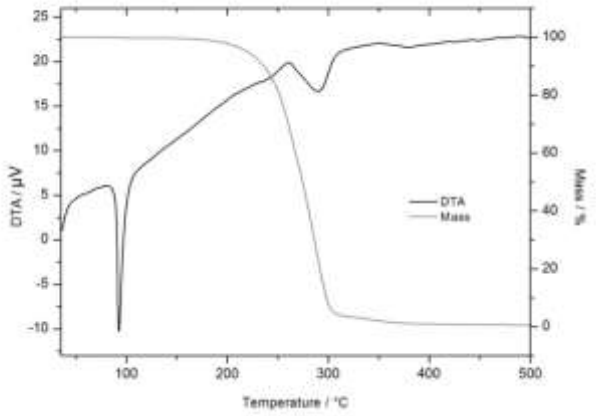

Fig-4: Thermo gravimetric curve (TGA) and differential thermal analysis (DTA) of LFQM117.

The results of solubility measurements in different vehicles are shown in Table 4. LFQM117 was practically insoluble in water (71.8 ng mL^{-1}). As previously discussed, LFQM117 is a non-ionic compound with a slight acid character. Like this, the solubility of this compound in phosphate buffer pH 5 was slightly lower than in water and the solubility in phosphate buffer pH 7.4 was practically the same observed in water. The LFQM117 solubility in phosphate buffer pH 7.4 with surfactants indicated that Poloxamer 407 has a minor effect in increasing the LFQM117 solubility in comparison with other evaluated surfactants. The surfactant Chemophor EL has a major effect in promoting LFQM117 solubility. During studies for cutaneous absorption evaluation, it is necessary a receptor phase in which the target compound has a good solubility, avoiding the saturation of the receptor phase with the drug. This way, phosphate buffer pH 7.4 with Chemophor EL at 10% m/v was selected as a receptor phase in skin permeation studies, assuring the sink conditions that are necessary during *in vitro* evaluations.

Table-4: LFQM117 solubility in different vehicles

Vehicle	Solubility ± SD (µg mL^{-1})
Water	0.07 ± 0.05
Isopropyl alcohol	0.57 ± 0.03
Phosphate buffer pH 5.0	0.05 ± 0.01
Phosphate buffer pH 7.4	0.07 ± 0.01
Phosphate buffer pH 7.4 + Tween 20® (5% m/v)	168.02 ± 8.06
Phosphate buffer p H7.4 + Tween 80 ® (5% m/v)	272.94 ± 25.01
Phosphate buffer pH 7.4 + SDS (5% m/v)	183.92 ± 6.20
Phosphate buffer pH 7.4 + Poloxamer 407 (5% m/v)	2.78 ± 0.19
Phosphate buffer pH7.4 + Cremophor EL® (10% m/v)	819.21 ± 92.96

SD, standard deviation; SDS, sodium dodecyl sulphate

According to the results in Table 4, it was supposed that LFQM117 is a lipophilic molecule. It was previously supposed by analysis of structural formulae of LFQM117. The theoretical value of *log P* obtained by ACD/I-Lab software (Version 6.0 for Microsoft Windows) was 5.5. The experimental *log P* of LFQM117 was obtained by an indirect HPLC method previously reported. The value found for *log P* was 4.8. This result is an important evaluation to be considered in the design of dermal formulations. In general, cutaneous penetration of lipophilic compounds is larger than the hydrophilic drugs, and a larger penetration into the skin layers is an indicative parameter of the efficacy of topical formulations.

Solubility and lipophilicity are key parameters in preformulation studies of topical formulations. From these measurements, a vehicle to drug incorporation can be selected. The selection of the vehicle is a fundamental step to obtain a formulation with stability and drug compatibility. In many cases, dispersions of the compound in an appropriate vehicle are a strategy to achieve the maximal thermodynamic activity, in that a major drug penetration is assured [25, 26]. Then, LFQM117 dispersion in mineral oil (hydrophobic vehicle) at 1% w/v was prepared. These dispersions with or without the chemical enhancer oleic acid were evaluated by *in vitro* studies using the vertical diffusion cell. *In vitro* studies allow predicting the *in vivo* behaviour of the formulations. For this, *in vitro* methodologies must simulate *in vivo* conditions, selecting an appropriate skin model, maintaining the

physiological temperature and avoiding the saturation of the receptor phase. Porcine ear skin has been recommended for evaluations in the vertical diffusion cell, once that this skin model can simulate the biochemical characteristics of the human skin [27]. The results from *in vitro* skin absorption (permeation + penetration) evaluations using porcine ear skin are shown in Table 5.

Table-5: Skin absorption of LFQM117 (penetration and retention) (n = 3)

Vehicle	Cumulative permeation ± SD (μg cm^{-2})			Penetration ± SD (μg cm^{-2})
	3 h	6 h	9 h	9 h
Mineral oil	0.25 ± 0.23	1.00 ± 0.69	14.56 ± 9.83	5.10 ± 2.66
Mineral oil + oleic acid (5% m/v)	9.15 ± 3.14	25.99 ± 18.76	73.54 ± 46.69	2.90 ± 0.62

SD, standard deviation.

Results indicated the skin absorption of LFQM117 from the oil dispersions (Table 5). In general, stratum corneum is an efficient barrier, and it limits the penetration of many compounds. Therefore, the development of topical formulations is fundamental work to assure an appropriate bioavailability of the compound into the skin. The effect of oleic acid was observed in these evaluations. According to variance analysis (ANOVA) followed by Tukey test (p=0.05), at 5% m/v, oleic acid increased significantly the LFQM117 permeation (p<0.05), but it did not increase the drug retention (p=0.0848). In this way, it was demonstrated that LFQM117 can penetrate the skin, and its percutaneous absorption can be modified from the appropriate design of the formulations.

CONCLUSION

In this work, a rapid, selective and sensible analytical method by reversed phase high performance liquid chromatography was developed and validated to be applied in preformulation studies of a novel benzophenone derivative denominated LFQM117 to topical formulations design. The method was suitable to LFQM117 determination in all assays of preformulation study. The preformulation studies showed that LFQM117 is stable and a lipophilic molecule with very low solubility in water. Skin absorption studies indicated that this drug candidate may penetrate into the skin, where it is expected to exert its pharmacological activity, demonstrating that it may be incorporated in topical pharmaceutical formulations.

ACKNOWLEDGEMENTS

The authors would like to thank Universidade Federal de Alfenas (Unifal-MG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their financial support.

The authors declare that there is no conflict of interest regarding the publication of this paper.

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