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An ultra-high performance liquid chromatography method to determine the skin penetration of an octyl methoxycinnamate-loaded liquid crystalline system

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Cutaneous penetration is a critical factor in the use of sunscreen, as the compounds should not reach systemic circulation in order to avoid the induction of toxicity. The evaluation of the skin penetration and permeation of the UVB filter octyl methoxycinnamate (OMC) is essential for the development of a successful sunscreen formulation. Liquid-crystalline systems are innovative and potential carriers of OMC, which possess several advantages, including controlled release and protection of the filter from degradation. In this study, a new and effective method was developed using ultra-high performance liquid chromatography (UPLC) with ultraviolet detection (UV) for the quantitative analysis of penetration of OMC-loaded liquid crystalline systems into the skin. The following parameters were assessed in the method: selectivity, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ). The analytical curve was linear in the range from 0.25 to 250 $\mu g.m^{-1}$, precise, with a standard deviation of 0.05–1.24%, with an accuracy in the range from 96.72 to 105.52%, and robust, with adequate values for the LOD and LOQ of 0.1 and 0.25 $\mu g.mL^{-1}$, respectively. The method was successfully used to determine the *in vitro* skin permeation of OMC-loaded liquid crystalline systems. The results of the *in vitro* tests on Franz cells showed low cutaneous permeation and high retention of the OMC, particularly in the stratum corneum, owing to its high lipophilicity, which is desirable for a sunscreen formulation.

1. Introduction

Sunscreens are used to protect the human skin from ultraviolet radiation (UV) produced by sunlight or artificial lights. They are able to prevent harmful effects, such as skin cancer and erythema, and retard skin aging (Marrot 2008; Shi et al. 2012). There is a great demand for systems with higher efficiency, chemical stability, and lack of toxicity (Ferreira et al. 2013). Therefore, it is very important that the sunscreen filter will be retained on the surface and upper layers of the skin to avoid systemic absorption (Mestres et al. 2010). To meet the requirements of filter retention in the epidermis, the physicochemical characteristics of the filter and formulation are both important.

Octyl methoxycinnamate (OMC), or 2-ethylhexyl-4-methoxycinnamate (EMC), is an organic liquid lipophilic UVB sunscreen (Fig. 1). In sunscreen, OMC can be used at concentrations of up to 10% and is usually combined with other sunscreens to increase the sun protection factor (SPF) of the final formulation (Chisvert et al. 2001; Mancebo et al. 2014). Currently, it represents one of the most widely used sunscreens with photoprotective formulations. However, OMC can cause toxicity, such as endocrine disruption, after percutaneous absorption; this is related to the hypothalamic release of gonadotropin-releasing hormone. In men, OMC stimulates the release of GABA, which decreases glutamate (GLU); whereas, in women, it decreases the excitatory amino acid aspartate (ASP) and GLU, but does not influence GABA release (Hayden et al. 1997; Sarveiya et al. 2004; Janjua et al. 2004; Axelstad et al. 2011; Christen et al. 2011; Klammer et al. 2007; Ozáez et al. 2013; Schlumpf et al. 2004; Carbone et al. 2010). Therefore, several studies have been conducted for the development of sunscreen formulations to reduce the cutaneous permeation of OMC and increase its retention in the skin (Jiménez et al. 2004; Puglia et al. 2014; Freitas et al. 2015).

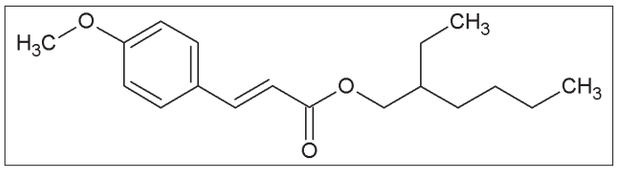

Fig. 1: Chemical structure of the filter OMC

Furthermore, many reports have indicated that the release, permeation, and retention of OMC were influenced by the physicochemical properties of the formulation used (Montenegro et al. 2011; Monteiro et al. 2012; Brinon et al. 1999). For example, Monteiro et al. (2012) compared liposomes and β-cyclodextrins as formulation components for OMC and concluded that liposomes were the most advantageous formulation because of their ability to increase the amount of OMC in the epidermis, owing to the similarity of the liposome with the lipids of the stratum corneum, which created an OMC reservoir and decreased the risk of percutaneous absorption. Because of the influence of the type of formulation on the skin absorption of OMC, the study of appropriate carriers for OMC incorporation is necessary, in addition to the evaluation of OMC skin penetration and retention.

Many sunscreen formulations have utilized nanotechnology in order to improve the performance of photoprotective agents

(Brinon et al. 1999; Puglia et al. 2014). In these formulations, the liquid-crystal systems (LCs) are nanostructured systems that feature liquid and solid characteristics, which are represented by the fluidity and the structure, respectively; they represent an intermediate state between solids and liquids, and are accordingly termed mesophases (Petrilli et al. 2016). These systems may offer advantages, such as greater retention in the skin and decreased skin permeation, the promotion of controlled release, and the protection of sunscreen from photodegradation (Brinon et al. 1999). Therefore, LC formulations are promising for sunscreen filters incorporation.

After considering the influence of the formulation on UV filter skin permeation, an adequate analytical method is needed for reliable filter quantification in the different layers of the skin during formulation development. Thus, several methods have been reported using high-performance liquid chromatography (HPLC) for the separation, detection, and quantification of sunscreens in different samples. However, few methods have been found for the identification and quantification of OMC. In general, studies have focused on the separation of the OMC from other sunscreens based on classic high-pressure liquid chromatography (HPLC) methods (Chisvert et al. 2001; Gaspar and Campos 2007; Sobanska and Pyzowski 2012). However, ultra-performance liquid chromatography (UPLC) is a relatively new technique, which advantageously allows the reduction of time and solvent consumption with improved resolution (Nováková et al. 2006). The advantages of UPLC are attributed to the use of short columns packed with smaller particles, which enable better separation compared with classic HPLC and the detection of analytes at very low concentrations, owing to improved signal-to-noise ratio (Klimczak and Gliszczyńska-Świgło 2015). To the best of our knowledge, the use of UPLC for the quantification of skin retention of OMC delivered from liquid crystalline formulations, which we have addressed in this study, has not been previously reported.

Thereby, the objective of this study was to develop an analytical method based on UPLC for the quantification of a novel OMC-loaded liquid crystalline formulation that permeated or was retained in different skin layers, which can be critical in the prediction of the safety and efficacy of new sunscreen formulations.

2. Investigations, results, and discussion

HPLC has been predominantly used in the majority of analytical procedures already reported for the quantification of OMC in sunscreen formulations or in skin samples (Vilela et al. 2011; Freitas et al. 2015). However, UPLC, widely used in the pharmaceutical industry, can be used for improved OMC quantification, enabling analyte quantification with better speed, resolution, and sensitivity owing to the use of column particle sizes below 2 µm. For example, UPLC has been shown to be more economical and possess superior chromatographic separation compared with HPLC for several analytes in different matrices; for example, the bulk powder and suspension dosage forms of posaconazole and vitamin C (Hamdy and Belal 2014; Klimczak et al., 2015). Given the important requirement for the development of an adequate, reliable, and sensitive method for sunscreen quantification in skin samples, as well as to ensure the safety of the sunscreen formulations (Vilela et al. 2011), the present study aimed to develop and assess an UPLC method to evaluate OMC skin retention and permeation from a liquid crystalline formulation.

The analytical curve was constructed from OMC values in the concentration range of 0.25–250 $\mu g \cdot mL^{-1}$. The method of least-squares regression was used to verify the linearity of the curve and the correlation coefficient (r) was 0.9999. Results above 0.99 and an accuracy between 96 and 101% within the range of 80–120% were obtained, which demonstrated that the analytical curve was in accordance with the ANVISA and FDA acceptance criteria; furthermore, the linear equation was y = 19307x - 571.3.

The chromatogram shows the optimal resolution of the chromatographic peak of OMC, which indicated the high selectivity and specificity of the method (Fig. 2). The retention time (t_R) of OMC was observed at 2.2 min; such a rapid time is considered an advantage for laboratory procedures. From the chromatograms, it was evident that there was no interference at the retention time of OMC from the components of the tape used to remove the stratum corneum or from the skin.

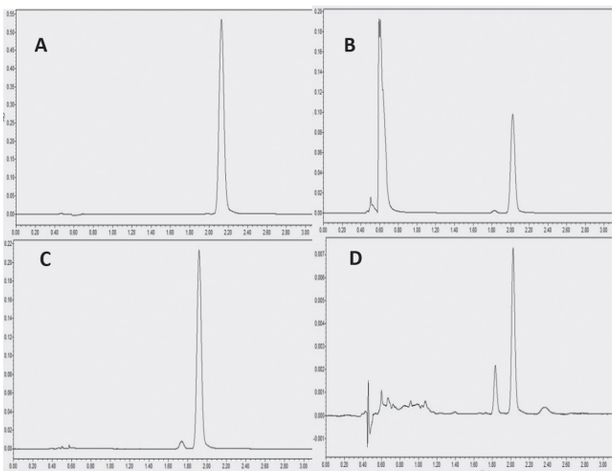

Fig. 2: Chromatogram of OMC obtained using an Acquity UPLC® HSS SB C18 column (2.1 × 100 mm; 1.8 µm) at 24 °C. The mobile phase was an acidic water:acetonitrile (20:80, v/v) mixture. The elution was performed in isocratic mode with detection at 310 nm. A flow rate of 0.5 mL/min was used and 2.0 µL of sample was injected. A, B, C, and D represent the chromatograms of OMC in standard solution, the stratum corneum extracted using the tape stripping method, the viable epidermis and dermis, and permeated OMC.

The accuracy and precision were determined in the same run, analyzed within a short period of time by the same analyst and the same instrumentation (intra run), and also on different days in three different runs (inter run) in three replicate concentrations: 200, 125, and 0.25 $\mu g \cdot mL^{-1}$, which provided quality control for high, medium, and low samples, respectively, expressed as a percentage. The accuracy is calculated as a percentage of the theoretical and experimental concentrations; the values obtained were in the range of 96.71–101.11 %. The results presented in Table 1 were within the acceptable range for the resolution (80–120 %). The precision was expressed as the relative standard deviation (RSD) and the values obtained were in the range of 0.05–5.49%. The results shown in Table 1 are within the acceptable range for resolution and did not exceed 5%, except for the lowest concentration, in which the obtained value was 5.49%.

Table 1: Intra-day and Inter-day accuracy and precision of quality control samples of OMC

	Intra-day (%)		Inter-day (%)	
Conc (µg/mL)	Accuracy	Precision	Accuracy	Precision
200	100.3	0.05	99.63	0.47
125	99.61	0.22	99.86	0.35
0.25	96.71	0.98	101.11	5.49

In this study, it was important to identify LQ and LD to determine the OMC concentration after *in vitro* assays. The lowest detectable concentration (LD) of the analytical method was 0.1 $\mu g \cdot mL^{-1}$, which was equivalent to three times the baseline noise. The lowest detectable concentration that showed an acceptable accuracy and precision of 0.25 $\mu g \cdot mL^{-1}$ and represented the lowest measurable concentration (LQ).

The robustness evaluates the ability of the method to remain unaffected when there are changes in the chromatographic conditions. For this study, the analysis was performed using a concentration of 200 $\mu g \cdot mL^{-1}$ and the following parameters were evaluated: column temperature, sample temperature, flow rate, ratio of mobile phase, pH of mobile phase, and column type. The values obtained are shown in Table 2, revealing that changes in the chromatographic

conditions resulted in acceptable accuracy and precision. Thus, the method was robust, except for a flow rate of 0.4 mL/min, which had an accuracy of 124.05 %, above the acceptable limit (80–120 %).

Table 2: Robustness of the analytical method for OMC quantification

Chromathografic conditions	Concentration found (µg/mL)	Accuracy (%)	Precision (%)
Column temperature			
22°C	198.61	99.30	0.07
26°C	198.44	99.22	0.90
Sample temperature			
21°C	197.02	98.51	0.58
25°C	196.95	98.47	0.94
Flow			
0.4 mL/min	248.11	124.05	0.28
0.6 mL/min	163.15	81.57	0.13
Ratio of mobile phase			
78%ACN/22%H_2O^-	197.12	98.56	0.26
82%ACN/18%H_2O^-	195.89	97.94	0.18
pH of mobile phase			
pH 2.8	200.80	100.40	0.32
pH 3.2	201.71	100.85	0.39
Type column			
HSS C18 SB (2.1x50 mm)	199.99	99.99	0.32

The analytical method was applied to determine the skin permeation and retention of OMC-loaded liquid crystalline systems. *In vitro* permeation and skin retention assays were performed using a pig ear as skin model. For this purpose, a liquid crystalline formulation that contained 5 % OMC (approximately 3000 µg·mL^{-1}) was prepared and analyzed. The samples were subjected to the test by using a Franz cell over a 12-h period. The skin retention results showed that the OMC concentration in the stratum corneum was high (315.3±13.08 µg·cm^2), whereas the retention in the dermis and epidermis was lower (approximately 75.52±23 µg·cm^2). Additionally, OMC skin permeation achieved a concentration of 1.38±34.35 µg·cm^2 after 12 h, which was equivalent to an approximately 230-fold lower than the concentration found in the stratum corneum; this may be predictive of the *in vivo* behavior (Fig. 3). The high standard deviation of permeated OMC could be explained by the low agitation speed used (300 rpm), although we followed a previous report (Christensen et al. 2011). In contrast, higher speed rates have been correlated with more reproducible results and lower standard deviations in *in vitro* studies (Klein 2013). However, it was evident that OMC was located in higher amounts in the stratum corneum, which is desirable, as sunscreen filters should remain on the skin surface and accumulate in the stratum corneum to create a barrier against UV radiation (Vilela et al. 2011).

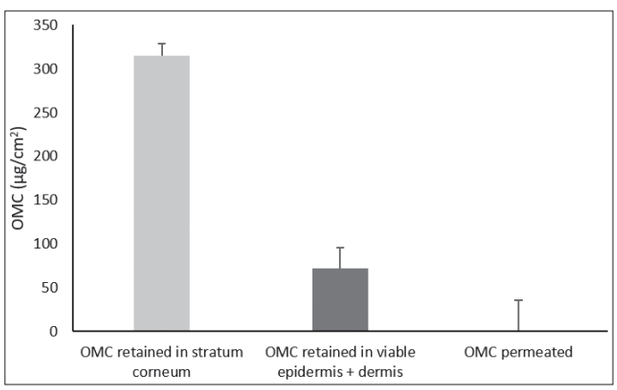

Fig. 3: OMC skin retention and permeation from a liquid crystalline formulation evaluated using the developed UPLC method.

Vilela et al., (2011) evaluated the cutaneous permeation and retention of UV filters and found that the majority of filters did not permeate, but were retained in the stratum corneum after 12 h with reduced delivery to the viable epidermis, in accordance with our observation. Furthermore, the retention in the stratum corneum and low permeation found in our studies are indicative that the formulation developed herein could meet the legislative requirements of sunscreen, which state that UV filters should not reach the systemic circulation (Latha et al. 2013).

The physicochemical characteristics of OMC can help explain its cutaneous penetration behavior. OMC has oil-water partition coefficient of 5.96, which is indicative of its high lipophilicity. Therefore, it is likely that OMC is able to accumulate and form a reservoir within the lipid phases of the stratum corneum. Conversely, OMC would have difficulty in the penetration of the viable epidermis owing to the hydrophilic nature of this layer (Vilela et al. 2011).

Moreover, it should be considered that the formulation might have exerted effects in the promotion of OMC retention into the skin, rather than permeation. The vehicle may have an impact on the UV filter skin retention and consequently on the sun protection efficacy and potential toxicological risks associated with skin permeation (Chatelain 2003). Vilela et al. (2011) compared different loading formulations of sunscreen filters and concluded that the cream and lotion formulations, which, owing to their higher lipophilic characteristics, were able to better retain lipophilic UV filters and do not readily penetrate the skin compared with the cream gel. Furthermore, liquid crystalline systems, which have been previously used as delivery systems of sunscreen filters, are promising OMC carriers and should be further evaluated with respect to their photoprotective effects (Manaia et al. 2015).

In conclusion, the UPLC-based analytical method reported herein was demonstrated to be selective, linear in the range of 0.25–200 µg·mL^{-1}, precise, accurate, robust, and with adequate limits of detection and quantification of 0.1 and 0.25 µg·mL^{-1}, respectively. Thus, the UPLC method enabled the successful quantification of OMC in different porcine skin layers and in the permeated fraction. Finally, liquid crystalline formulations were shown to be effective for localizing OMC into upper layers of the skin, particularly in the stratum corneum, which is likely to result in minor toxicity.

3. Experimental

3.1. Chromatographic conditions

A Waters Acquity UPLC® system equipped with UV-Vis detector was used. The chromatographic separation was performed on an Acquity UPLC® HSS SB C18 column (2.1 × 100 mm; 1.8 µm) at 24 °C. The mobile phase was an acidic water:acetonitrile (20:80, v/v) mixture, and elution was performed in isocratic mode with detection at 310 nm. A flow rate of 0.5 mL/min was used and 2.0 µL of sample was injected into the chromatographic system. The sample auto-injector was maintained at 23 °C and the run time was 3.5 min.

3.2. Preparation of standard solutions

The standard stock solution of OMC (250 mg·mL^{-1}) was prepared in isopropanol and stored at 4 ºC in a light-protected bottle. Appropriate serial dilutions of the stock solutions were prepared in isopropanol (J. T. Baker, USA) to produce a range of OMC concentrations from 0.1 to 250 µg·mL^{-1}. The standard curve was constructed using 13 concentrations of the area of OMC.

3.3. UPLC method assessment

The method followed the "Guide for validation of analytical and bioanalytical methods" according to resolution 899 of Brazilian National Health Surveillance Agency (ANVISA), which determines parameters for analytical method validation, such as selectivity, linearity, accuracy, precision, and their acceptance criteria (Brazil 2003).

3.3.1. Analytical curve and linearity

The calibration curve was generated from the OMC peak area via least-squares linear regression. The concentration levels were prepared as serial dilutions of the calibration curve in the range of 0.25–250 µg·mL^{-1}. ANOVA (analysis of variance) ($p<0.05$) was used to test the linearity of the calibration curve. The samples were prepared and analyzed in triplicate. The analysis of the main curve by least-squares regression requires a minimum correlation coefficient (r) of 0.99 to be acceptable for linearity.

3.3.2. Specificity and selectivity

Specificity was demonstrated by comparing chromatograms of samples that contained a known quantity of OMC (standard solution, stratum corneum removed with tape stripping, and viable epidermis and dermis) with blank samples (without OMC).

3.3.3. Accuracy and precision

Precision and accuracy were assessed for three different concentrations at 200, 125, and 0.25 $\mu g \cdot mL^{-1}$ (high QC, mid QC, and low QC), in three replicates. The accuracy was defined as the deviation of the mean analytical result from the theoretical value, whereas precision was represented by the relative standard deviation (RSD) of measurements at each level. Intra-run precision and accuracy were calculated for three replicates on the same day. Inter-run precision and accuracy were calculated for six replicate determinations at each QC level analyzed over two non-consecutive days. The data acceptance criterion for precision was an RSD less than 5%. To confirm accuracy, the acceptable values were in the range of 80–120% of the theoretical value.

3.3.4. Limit of detection and limit of quantitation

The limit of detection (LD) and limit of quantitation (LQ) were determined from the calibration curve. The LD represented the lowest detectable concentration of OMC and the LQ represented the lowest concentration of OMC with acceptable precision and accuracy.

3.3.5. Robustness

The robustness was assessed by evaluating the system after changes in the column temperature (22 °C and 26 °C), sample temperature (21 °C and 25 °C), flow rate (0.4 and 0.6 mL/min), composition ratio of mobile phase (78% ACN/22% H_2O and 82% ACN/18% H_2O), pH of mobile phase (2.8 and 3.2), and type of column, HSS C18 SB (2.1 × 50 mm) and HSS SB C18 column (2.1 × 100 mm; 1.8 µm). The method was assessed for accuracy and precision.

3.4. Preparation of the liquid crystalline model formulation for OMC

The aqueous phase of the LC formulation, which represented 20% of the total weight, was composed by 0.5% polycarbophil - Noveon® AA-1 (Lubrizol, USA), prepared in water by stirring for 10 min. Furthermore, the pH of the aqueous solution was adjusted to 7.0 with triethanolamine (Deg, Brazil). The oily phase was prepared by mixing with 35% of the total weight of silicon, glycol copolymer fluids - DC® 193 (Dow Corning, USA), and 40% of surfactant (cetyl alcohol ethoxylated propoxylated - Procetyl® AWS). Then, OMC (Deg, Brazil) was added at a concentration of 5% in the oily phase and mixed using a pestle and mortar. Finally, the oily phase was added to aqueous phase and mixed with a pestle and mortar for 5 min.

3.5. OMC in vitro skin permeation and retention from a liquid crystalline formulation

The cutaneous permeation and retention tests were performed *in vitro* using Franz diffusion cells, in Hanson MicroettePlus® equipment (Chatsworth, USA) that used dermatomized pig ear skin. The cells were filled with 7.0 mL phosphate buffer (pH 7.4) that contained 2.0 % polysorbate 80. This receiving solution was stirred and heated (300 rpm at 32±0.5 °C), as previously reported (Christensen et al. 2011). After hydration with the receptor solution, the skin was placed on the diffusion cell and 400 mg of sample was placed above the skin. The experiment was conducted for 12 h and the samples were withdrawn after 1, 2, 4, 6, 8, 10, and 12 h by using a Hanson AutoPlus™ Multifill™ autosampler (Chatsworth, USA).

After the skin penetration and permeation tests, the skin was removed from the Franz cell and the tape stripping technique was performed to remove the stratum corneum by the consecutive application of adhesive tape. The tapes were placed in assay tubes that contained 3 mL isopropanol, vortexed for 2 min, and sonicated for 30 min. The supernatant was then filtered and analyzed via UPLC for the quantification of OMC retained in the stratum corneum. Thereafter, the part of the skin that was in contact with the formulation was cut and perforated to assess the amount of OMC retained in the dermis and epidermis. The skin was placed in assay tubes that contained 3 mL isopropanol, vortexed for 2 min, homogenized using an Ultraturrax for 1 min, sonicated for 30 min, and centrifuged for 7 min at 3,000 rpm. Then, the samples were filtered and the supernatant was analyzed via UPLC for the quantification of OMC retained in viable epidermis and dermis. In addition, the aqueous medium in the recipient compartment was analyzed by UPLC to quantify permeated OMC (Touitou et al. 1998).

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Conflicts of interest: None declared.

References

Axelstad M, Boberg J, Hougaard KS, Christiansen S, Jacobsen PR, Mandrup KR, Nellemann C, Lund SP, Hass U (2011) Effects of pre- and postnatal exposure to the UV-filter octyl methoxycinnamate (OMC) on the reproductive, auditory and neurological development of rat offspring. Toxicol Applied Pharmacol 250: 278–290.

Brazil (2003) Guide for validation of analytical and bioanalytical methods Resolution 899 of Brazilian National Health Surveillance Agency (ANVISA).

Brinon L, Geiger S, Alard V, Doucet J, Tranchant JF, Couarraze G (1999) Percutaneous absorption of sunscreens from liquid crystalline phases. J Control Release 60: 67–76.

Carbone S, Szwarcfarb B, Reynoso R, Ponzo OJ, Cardoso N, Ale E, Moguilevsky JA, Scacchi P (2010) In vitro effect of octyl methoxycinnamate (OMC) on the release of Gn-RH and amino acid neurotransmitters by hypothalamus of adult rats. Exp Clin Endocrinol Diabetes 118: 298–303.

Chatelain EB, Gabard, Surber C (2003) Skin penetration and sun protection factor of five UV filters: effect of the vehicle. Skin Pharmacol Applied Skin Physiol 16: 28–35.

Chisvert A, Pascual-Martí MC, Salvador A (2001) Determination of the UV filters worldwide authorised in sunscreens by high-performance liquid chromatography: use of cyclodextrins as mobile phase modifier. J Chromatog A 921: 207–215.

Christen V, Zucchi S, Fent K (2011) Effects of the UV-filter 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) on expression of genes involved in hormonal pathways in fathead minnows (Pimephales promelas) and link to vitellogenin induction and histology. Aquatic Toxicol 102: 167–q76.

Christensen JM (2011) Hydrocortisone diffusion through synthetic membrane, mouse skin, and epiderm cultured skin. Arch Drug Inform 4: 10–21.

Ferreira VS (2013) Voltammetric analysis of sun-block preparations containing octocrylene and its association with 2-hydroxy-4-methoxybenzophenone and octyl methoxycinnamate. Microchem J 106: 378–383.

Freitas JV, Peporine Lopes N, Rigo Gaspar L (2015) Photostability evaluation of five UV-filters, trans-resveratrol and beta-carotene in sunscreens. Eur J Pharm Sci 78: 79–89.

Gaspar LR, Maia Campos PMBG (2007) Photostability and efficacy studies of topical formulations containing UV-filters combination and vitamins A, C and E. Int J Pharm 343: 181–89.

Hamdy DA, Belal TS (2014) A comparative study of newly developed HPLC-DAD and UHPLC-UV assays for the determination of posaconazole in bulk powder and suspension dosage form. J Anal Methods Chem 2014: 241035.

Hayden CGJ, Roberts MS, Benson HAE (1997) Systemic absorption of sunscreen after topical application [4]. Lancet 350: 863–864.

Janjua NR, Mogensen B, Andersson AM, Petersen JH, Henriksen M, Skakkebaek NE, Wulf HC (2004) Systemic absorption of the sunscreens benzophenone-3, octylmethoxycinnamate, and 3-(4-methyl-benzylidene) camphor after whole-body topical application and reproductive hormone levels in humans. J Invest Dermatol 123: 57–61.

Jiménez MM, Pelletier J, Bobin MF, Martini MC (2004) Influence of encapsulation on the in vitro percutaneous absorption of octyl methoxycinnamate. Int J Pharm 272: 45–55.

Klammer H, Schlecht C, Wuttke W, Schmutzler C, Gotthardt I, Köhrle J, Jarry H (2007) Effects of a 5-day treatment with the UV-filter octyl-methoxycinnamate (OMC) on the function of the hypothalamo-pituitary-thyroid function in rats. Toxicology 238: 192–199.

Klein S (2013) Influence of different test parameters on in vitro drug release from topical diclofenac formulations in a vertical diffusion cell setup. Pharmazie 68: 565–571.

Klimczak I, Gliszczyńska-Świgło A (2015) Comparison of UPLC and HPLC methods for determination of vitamin C. Food Chem 175: 100–105.

Latha MS (2013) Sunscreening agents: a review." J Clin Aesthetic Dermatol 6: 16–26.

Manaia EB, Kaminski RC, de Oliveira AG, Corrêa MA, Chiavacci LA (2015) Multifunction hexagonal liquid-crystal containing modified surface TiO_2 nanoparticles and terpinen-4-ol for controlled release. Int J Nanomed 10: 811–819.

Mancebo, SE, Hu, JH, Wang, SQ. (2014). Sunscreens: a review of health benefits, regulations and controversies. Dermatol Clin. 32:427–438.

Marrot L, Meunier JR (2008) Skin DNA photodamage and its biological consequences. J Am Acad Dermatol 58 (5 Suppl. 2): 139–148.

Mestres JP, Duracher L, Baux C, Vian L, Marti-Mestres G (2010) Benzophenone-3 entrapped in solid lipid microspheres: formulation and in vitro skin evaluation. Int J Pharm 400: 1–7.

Monteiro MS, Ozzetti RA, Vergnanini AL, de Brito-Gitirana L, Volpato NM, de Freitas ZM, Ricci-Júnior E, dos Santos EP (2012) Evaluation of octyl p-methoxycinnamate included in liposomes and cyclodextrins in anti-solar preparations: preparations, characterizations and in vitro penetration studies. Int J Nanomed 7: 3045–3058.

Montenegro L, Carbone C, Puglisi G (2011) Vehicle effects on in vitro release and skin permeation of octylmethoxycinnamate from microemulsions. Int J Pharm 405: 162–168.

Nováková L, Matysová L, Solich P (2006) Advantages of application of UPLC in pharmaceutical analysis. Talanta 68: 908–918.

Ozáez I, Martínez-Guitarte JL, Morcillo G (2013) Effects of in vivo exposure to UV filters (4-MBC, OMC, BP-3, 4-HB, OC, OD-PABA) on endocrine signaling genes in the insect Chironomus riparius. Sci Total Environm 456–457: 120–126.

Petrilli R, Eloy JO, Praça FS, Del Ciampo JO, Fantini MA, Fonseca MJ, Bentley MV (2016) Liquid crystalline nanodispersions functionalized with cell-penetrating peptides for topical delivery of short-interfering RNAs: a proposal for silencing a pro-inflammatory cytokine in cutaneous diseases. J Biomed Nanotechnol 12: 1063–1075.

Puglia C, Damiani E, Offerta A, Rizza L, Tirendi GG, Tarico MS, Curreri S, Bonina F, Perrotta RE (2014) Evaluation of nanostructured lipid carriers (NLC) and nanoemulsions as carriers for UV-filters: characterization, in vitro penetration and photostability studies. Eur J Pharm Sci 51: 211–217.

Sarveiya V, Risk S, Benson HAE (2004) Liquid chromatographic assay for common sunscreen agents: application to in vivo assessment of skin penetration and systemic absorption in human volunteers. J Chromatogr B 803: 225–231.

Schlumpf M (2004) Endocrine activity and developmental toxicity of cosmetic UV filters - an update. Toxicology 205 (1–2 Spec. Iss.): 113–122.
Shi L, Shan J, Aikens P, Prud'homme RK (2012) Nanoparticles as delivery vehicles for sunscreen agents.Colloids Surfaces A 396: 122–129.
Touitou E, Meidan VM, Horwitz E (1998) Methods for quantitative determination of drug localized in the skin. J Control Release 56: 7–21.
Vilela FMP, Fonseca YM, Vicentini FTMC, Fonseca MJV, da Penha Henriques do Amaral M (2011) Determination of three ultraviolet filters in sunscreen formulations and from skin penetration studies by high-performance liquid chromatography. Quimica Nova 34: 879–883.