International Journal of Pharmaceutics 510 (2016) 250–254

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Rapid communication

Chemical ultraviolet absorbers topically applied in a skin barrier mimetic formulation remain in the outer stratum corneum of porcine skin

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ARTICLE INFO

Article history:
Received 2 May 2016
Received in revised form 15 June 2016
Accepted 16 June 2016
Available online 16 June 2016

Keywords:
Sunscreens
Mass balance
Permeation studies
Porcine skin
Tape stripping
Topical

ABSTRACT

The objective of the present study was to evaluate the fate of three chemical sunscreens, isoamyl *p*-methoxycinnamate (IPMC), diethylamino hydroxybenzoyl hexyl benzoate (DHHB), and bis-ethyl-hexylphenol methoxyphenyl triazine (BEMT), topically applied to mammalian skin from a skin barrier mimetic oil-in-water formulation. High Performance Liquid Chromatography (HPLC) methods were developed for the analysis of each molecule and validated. Franz cell permeation studies were conducted following application of finite doses of the formulations to excised porcine skin. A vehicle formulation containing no sunscreens was evaluated as a control. Permeation studies were conducted for 12 h after which full mass balance studies were carried out. Analysis of individual UV sunscreens was achieved with HPLC following application of the formulation to the skin with no interference from the vehicle components. No skin permeation of any of the chemical sunscreens was evident after 12 h. While sunscreens were detected in up to 12 tape strips taken from the SC, 87% or more of the applied doses recovered in the first 5 tape strips. When corrected for the amount of protein removed per tape strip this corresponded to a penetration depth in porcine stratum corneum of ~1.7 μm. Mass balance studies indicated total recovery values were within accepted guidelines for cosmetic formulations. Overall, only superficial penetration into the SC was observed for each compound. These findings are consistent with the physicochemical properties of the selected UV absorbing molecules and their formulation into an ordered biomimetic barrier formulation thus support their intended use in topical consumer formulations designed to protect from UV exposure. To our knowledge this is the first report of depth profiling of chemical sunscreens in the SC that combines tape stripping and protein determination following *in vitro* Franz cell studies.

1. Introduction

Ultraviolet (UV) light has both positive and negative effects on human skin depending on the nature of the radiation and duration of exposure. Exposure of humans to UV radiation is essential for normal production of vitamin D and it is also used in the management of rickets, eczema, psoriasis and jaundice. However, UV may also cause degenerative changes in skin cells, ultimately resulting in skin aging, erythema, photodermatoses, actinic keratosis and skin cancer (Leigh, 2014). UV radiation is further classified based on specific wavelength ranges of the electromagnetic spectrum, namely UVA (320–400 nm), UVB (280–320 nm) and UVC (100–280 nm). UVA radiation may be subdivided further as UVA1 (340–400 nm) and UVA2 (320–340 nm). UVC or short wave radiation is absorbed by ozone and attenuated by the atmosphere and does not normally cause significant irradiation of humans. UVB radiation can only penetrate the superficial layers of the skin and may cause tanning, burning, photo-aging and cancer of the skin. UVA is the most available radiation (95%) on earth's surface and penetrates the deep layers of the skin. It may also induce tanning effects, skin aging, wrinkle formation and skin cancers (Marionnet et al., 2014; D'Orazio et al., 2013).

Skin protection against harmful UV radiation may be achieved by topical application of (i) physical barriers which deflect and scatter radiation or (ii) actives which absorb UV

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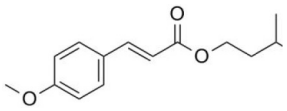
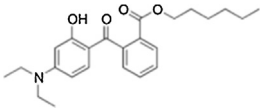
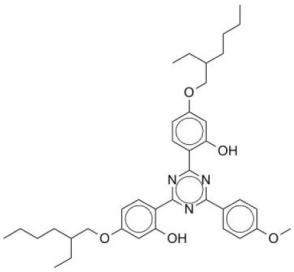
http://dx.doi.org/10.1016/j.ijpharm.2016.06.041
0378-5173/

Table 1
Experimental and predicted physicochemical parameters of isoamyl *p*-methoxycinnamate (IPMC), diethylamino hydroxybenzoyl hexyl benzoate (DHHB) and bis-ethylhexyloxyphenol methoxyphenol triazine (BEMT).

	Isoamyl *p*-methoxycinnamate	Diethylamino hydroxybenzoyl hexyl benzoate	Bis-ethylhexyloxyphenol methoxyphenol triazine
Chemical structure			
Brand name	Neo Heliopan® E1000	Uvinul® A Plus	Tinosorb® S
Molecular weight (Da)	248.3	397.5	627.8
Log P	3.6[a]	5.7[a] 6.2[b]	12.6[a] >5.7[c]
Water solubility (mg/L)	4.9 (25 °C)[d]	<0.01 (20 °C)[b]	$<10^{-4}$[c]
Melting point (°C)	N/A	54; 314 (decomposition temperature)[b]	80.40 ± 0.10[c]

[a] Calculated with ChemBioDraw®.
[b] Scientific Committee on Consumer Products (2008).
[c] Ruiz (2000).
[d] The Environment Agency (2008).

radiation. Titanium dioxide (TiO_2) and zinc oxide (ZnO) are the most commonly used materials in physical barriers whereas a much larger range of compounds are available for use as chemical UV absorbers (Skotarczak et al., 2015). Although a number of *in vitro* studies have examined the interaction of TiO_2 and ZnO particles with skin, comparatively fewer studies have been reported for chemical sunscreens (Gamer et al., 2006; Cross et al., 2007; Senzui et al., 2010). Knowledge of the fate of these materials following application to the skin is important in order to ensure that they are effective and that their residence time is adequate to assure UV protection. The aim of the present work was, therefore, to evaluate the skin disposition of three UV absorbers *in vitro* following application in a barrier mimetic oil-in-water topical formulation. The specific UV absorbers selected for study were isoamyl *p*-methoxycinnamate (IPMC), diethylamino hydroxybenzoyl hexyl benzoate (DHHB) and bis-ethylhexyloxyphenol methoxyphenol triazine (BEMT). These three molecules were evaluated because they span a range of molecular weights and physical states (Table 1) and when formulated in combination provide protection from UVB and UVA radiation. IPMC (also known as amiloxate) is an efficient UVB absorber and is a liquid at room temperature. It is a lipophilic molecule and the maximum amount used in topical formulations is 10% (Couteau et al., 2007; Environment Agency, 2008). DHHB is a white to light salmon coloured powder and is an oil soluble UVA filter. It may be used alone or in combination with other UV filters to a maximum amount of 10% (European Commission, 2008). BEMT (also known as bemotrizinol) is a broad spectrum UV absorber which is effective against both UVA and UVB radiation. In appearance, it is a light yellow powder and is typically incorporated in formulations at a level of 5%, although it is approved for use up to 10% (Ruiz, 2000). We also report a new HPLC method for analysis of each molecule. Finally, the overall distribution of each of the UV absorbers, in and on the skin, is accounted for, via a mass balance approach.

2. Materials and methods

2.1. Materials

IPMC (NeoHeliopan® E1000), DHHB (Uvinul® A Plus) and BEMT (Tinosorb® S) were provided by GSK. The three sunscreens were incorporated in a biomimetic lamellar oil-in-water (o/w) formulation at amounts (w/w) typically used in personal care products and the same formulation without sunscreens was used as a control. The formulation also contained the following materials: hydrogenated lecithin, capric caprylic triglyceride, shea butter, glycerine, olus oil, isostearyl isostearate, dicapryl carbonate, xylitol, panthenol, niacinamide, pentylene glycol and 1,2 hexanediol. HPLC grade unstabilised tetrahydrofuran (THF), acetonitrile (ACN), and trifluoroacetic acid (TFA) were obtained from Fisher Scientific. Water and Brij™ 98 were purchased from (Sigma Aldrich, Dorset, UK). Phosphate buffered saline (PBS) was prepared by dissolving PBS tablets (Dulbecco A, Oxoid Limited, UK) in deionised water (pH 7.4 ± 0.2).

2.2. Methods

2.2.1. Analysis of chemical sunscreens

All UV filters were analysed using an Agilent 1200 HPLC system consisting of an Agilent G1322A degasser, Agilent G1311A quaternary pump, Agilent 1329A auto sampler, Agilent G1316A thermostat column compartment and Agilent G1314B UV absorbance detector (Agilent Technologies, Cheadle, United Kingdom). Separation of the molecules was performed using a Capcell Pak® C_{18} column (type MG II, 4.6 mm × 250 mm) purchased from Shiseido Ltd. (Tokyo, Japan). A gradient mobile phase method was developed and the solvent compositions and run times for each gradient stage are shown in Table 2. The column temperature was set at 40 °C and the flow rate and injection volume were 1.2 mL/min and 10 μL, respectively. Calibration curves were constructed by dissolving known amounts of the sunscreens in THF from 0.01 to 100 μg/mL.