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Liquid chromatographic assay for common sunscreen agents: application to in vivo assessment of skin penetration and systemic absorption in human volunteers

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Abstract

The purpose of the present study was to develop a reverse-phase high-performance liquid chromatographic (HPLC) assay for quantifying four common sunscreen agents, namely 2-hydroxy-4-methoxybenzophenone, 2-ethylhexyl-*p*-methoxycinnamate, 2-ethylhexylsalicylate (octylsalicylate) and salicylic acid 3,3,5-trimethcyclohexyl ester (homosalate) in a range of biological matrices. This assay was further applied to study the skin penetration and systemic absorption of sunscreen filters after topical application to human volunteers. Separation was achieved utilizing a Symmetry C_{18} column with methanol–water as the mobile phase. The assay permits analysis of the sunscreen agents in biological fluids, including bovine serum albumin (BSA) solution, plasma and urine, and in human epidermis. The assay was linear ($r^2 > 0.99$) with minimum detectable limits of 0.8 ng for oxybenzone, 0.3 ng for octylmethoxycinnamate, and 2 ng for homosalate and octylsalicylate. The inter- and intra-day variation for the four sunscreens was less than 3% at the upper end of the linear range and less than 6% at the lower end. Recoveries of sunscreens from plasma, 4% (w/v) BSA solution and epidermal membranes were within the range of 91–104%. Recoveries from urine of the four sunscreens, and oxybenzone with its metabolites were more than 86%. Up to approximately 1% of the applied dose of oxybenzone and its metabolites was detected in the urine. Appreciable amounts were also detected in the stratum corneum through tape stripping. The HPLC assay and extraction procedures developed are sensitive, simple, rapid, accurate and reproducible. Results from the preliminary clinical study demonstrate significant penetration of all sunscreen agents into the skin, and oxybenzone and metabolites across the skin.

Keywords: Skin penetration; Sunscreen agents

1. Introduction

The use of sunscreens has increased with the awareness of the detrimental effects of sun exposure on human skin such as erythema, skin aging and cancers. Sunscreen products are formulated to provide a specific sun protection factor (SPF) and to absorb a broad spectrum of ultraviolet radiation (UVR). In addition to traditional sunscreen products, sunscreen chemicals are also incorporated into a wide range of everyday hair and skin products and may therefore be used without the wearer making a conscious decision to apply a sunscreen.

The actives used in topical formulations are generally classified as either chemical or physical sunscreens. Physical sunscreens comprise of particles that act by scattering, reflecting, or absorbing the passage of radiation. Chemical sunscreens act by absorbing incident UVR and then dissipating it as longer wavelength energy, thereby protecting the skin from potentially damaging UVR. The efficiency of sunscreens is estimated by the sun protection factor, which depends on the content of UV filters in the formulation. The necessity to provide high SPF and screening efficiency against both UV-A (320–400 nm) and UV-B (290–320 nm) wavelengths has led to the development of sunscreen

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preparations containing many different sunscreen chemical combinations. Benzophenones, dibenzoylmethanes and anthranilates are the most common UV-A filters, whereas the UV-B filters include *p*-aminobenzoic acid (PABA) derivatives, salicylates, cinnamates, digalloyl triolate, lawsone, acrylates and benzimidazole derivatives. Of the approved sunscreen chemicals, oxybenzone (OX, benzophenone-3), octylmethoxycinnamate (OMC), butylmethoxydibenzoylmethane (BDM), octylsalicylate (OS) and homosalate (HS) are some of the most common active ingredients used in sunscreen formulations.

Recent studies have provided evidence that some sunscreens are absorbed systemically following topical application to the skin [1–3]. These studies involved determination in skin layers only or measurement of urinary excretion of absorbed sunscreens and their metabolites. Neither provided a full pharmacokinetic analysis, as only a single measure of absorption or excretion was assessed. It would be advantageous to quantify penetration within the skin tissue and systemic distribution of sunscreen agents following topical application. This would aid in the determination of the exposure of viable tissues to sunscreen chemicals, provide a better understanding of the potential for toxicity both locally and systemically, and facilitate design of novel formulations to target the outer skin layers.

In addition to in vivo studies, skin penetration of chemicals and drugs is frequently investigated using in vitro techniques. The in vitro technique utilizes diffusion cells, which consist of a receptor and donor phase separated by a synthetic or skin membrane. Where lipophilic solutes are investigated, as is the case for many sunscreens, bovine serum albumin (BSA) or other solubility modifiers are used as receptor fluids to provide adequate solubility and ensure sink conditions [4,5]. A suitable extraction procedure and high-performance liquid chromatographic (HPLC) assay is required to facilitate these studies.

Many of the HPLC assays published for sunscreen agents are designed for product evaluation and determination of concentrations in cosmetic formulations [6–8]. Few assays for evaluation of sunscreens in biological samples have been reported [5,9–11]. A reliable analytical method for the quantitative determination of the common sunscreen chemicals in biological fluids will facilitate the evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetic data.

The aim of this study was to develop simple, rapid and reliable operating procedures for quantification of sunscreen chemicals in a range of biological matrices. Butylmethoxydibenzoylmethane, octylmethoxycinnamate, octyldimethyl PABA, octylsalicylate, oxybenzone and homosalate are the most common sunscreen agents. Most of them present similar retention times in previously published assays and are therefore difficult to resolve. HS is especially problematic because it presents two peaks corresponding to two isomeric forms [6]. This paper provides a reproducible and accurate assay, by which four of the most common sunscreen agents

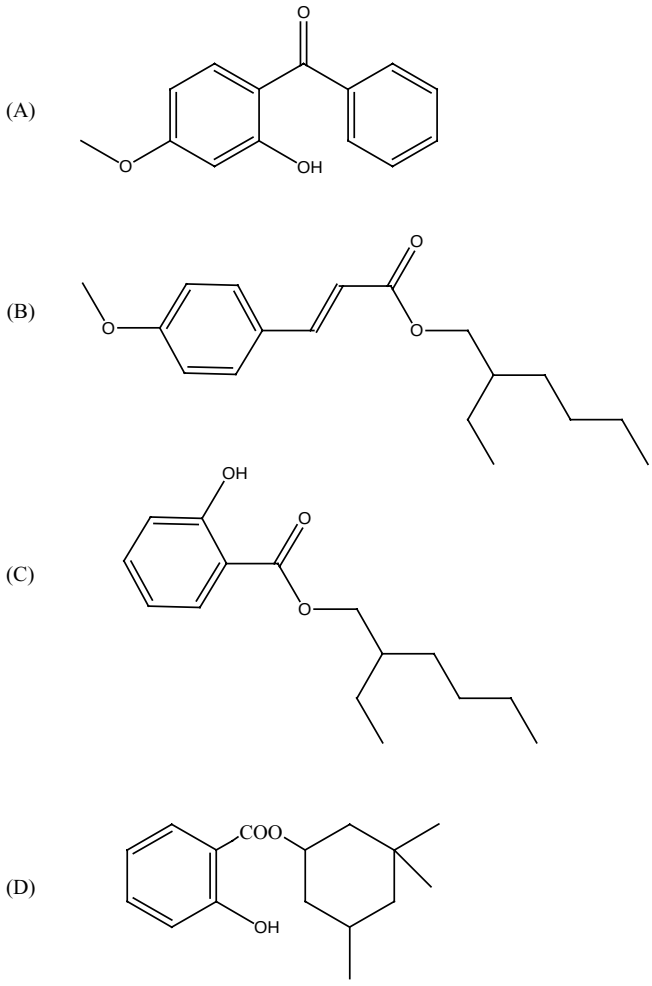

Fig. 1. Structures of the sunscreen agents: (A) oxybenzone; (B) octylmethoxycinnamate; (C) octylsalicylate; (D) homosalate.

(Fig. 1), including HS, can be resolved simultaneously. Using this assay procedure, a preliminary investigation of the penetration into the skin tissues, plasma and excretion in the urine of four common sunscreens, as active ingredients in a commercially available sunscreen product, was studied. This paper also provides procedures for the extraction of sunscreens from tape strips, skin tissue and biological matrices including plasma, urine and bovine serum albumin.

2. Experimental

2.1. Materials and methods

OX, OS and BSA were purchased from Sigma–Aldrich (USA). OMC and HS were gifts from BASF Corporation (NJ, USA) and EM Industries (Germany), respectively. Coppertone sunblock lotion (Schering-Plough Health Care Products Inc.) was the commercially available sunscreen product used for the study. HPLC grade methanol was from Fisher Scientific (USA). De-ionized water (Milli-Q, Waters Inc.,

USA) was used and all other chemicals used were analytical reagent grade.

2.2. HPLC instrumentation and conditions

An Alliance liquid chromatographic system (Waters Inc.) equipped with a 2690 separations module and 996 photodiode array detector was used. Separation was achieved at ambient temperature on a Symmetry C_{18} column (5 μm, 3.9 mm × 150 mm i.d., Waters Inc.) with an inline pre-filter. Integration was undertaken using a personal computer equipped with Millenium 4.0 version software.

The mobile phase consisted of methanol–water, filtered through a 0.45 μm membrane filter (Durapore, Millipore, USA). Gradient flow from 75:25 methanol–water to 92:8 methanol–water was used from 0 to 4 min, thereafter the flow was isocratic with 92:8 methanol–water. The solvent composition was returned to initial conditions after 11 min. The mobile phase was continuously degassed before and during use. The flow rate was 1.0 ml/min. To obtain a satisfactory UV response for all the analytes, each chemical was measured at its wavelength of maximum absorbance: oxybenzone at 289 nm, octylmethoxycinnamate at 310 nm, homosalate and octylsalicylate at 237 nm. Injection volumes of 10 μl were used for the assay.

Stock solutions were prepared by accurately weighing the agents (OX, OS, OMC and HS) and dissolving in methanol. Three working solutions of the four sunscreens were freshly prepared from their stock solutions by 1:10 dilution. Appropriate dilution of these working solutions gave concentrations of 0.1–0.5 μg/ml. The entire procedure was repeated on six different days to test inter-day variation and repeated six times at low and high concentrations to test intra-day variation.

The minimum detectable limits were measured by diluting the sunscreen agents with methanol to give an appropriate range from 0.01 to 20 μg/ml. Aliquots of 10 μl of the samples were injected onto the HPLC column.

2.3. Sample treatment and preparation

2.3.1. Plasma and BSA

The four sunscreen standards were spiked into human plasma and 4% (w/v) BSA in phosphate buffer (pH 7.4) at low and high concentrations (0.5 and 5.0 μg/ml). The sample solutions were stirred for 30 min following spiking to ensure complete dissolution of the sunscreen agents. After protein precipitation with two sample volumes of acetonitrile (200 μl acetonitrile to 100 μl of sample), the samples were centrifuged at 10,000 × *g* for 10 min. The supernatant was injected onto the HPLC system. Six replicates were performed at each concentration. Blank plasma and BSA in phosphate buffer were treated identically and injected onto the HPLC system to ensure that there were no peaks interfering with the sunscreen active substances.

2.3.2. Urine

The four sunscreen standards were spiked (5 μg/ml) into fresh human urine. To 1 ml of this solution was added an equal volume of phosphate buffer (pH 6.8). The sample was then treated with beta-glucuronidase enzyme (600 units) and heated at 37 °C for 24 h. The reaction was stopped with an equal volume of acetonitrile resulting in protein precipitation. It was vortex mixed and centrifuged at 5000 × *g* for 10 min. The sample was evaporated to dryness then re-suspended in methanol and 20 μl of the supernatant was then injected onto the HPLC column.

2.3.3. Skin tissue

Human epidermal membranes were immersed in 5 ml of standard sunscreen solution for 24 h in dark conditions at 25 °C. The formulation residue was removed from the epidermal tissue by rinsing with 5 ml distilled water three times and drying. Retained sunscreen was then extracted with 2 ml of absolute methanol for 30 min. The extraction procedure was repeated three times with methanol. After centrifuging each extract at 10,000 × *g* for 10 min, the resultant supernatants were diluted appropriately and quantified by HPLC.

2.4. Preliminary investigation of in vivo absorption of sunscreen

Ethical approval was obtained from the Health Research Ethics Board at the University of Manitoba and St. Boniface Hospital Ethics Committee. A commercially available sunscreen product, Coppertone Colorblok for kids (SPF 30) was applied at a dose of 2 mg/cm^2 to the arms and back of three female human volunteers, aged 22–42 years. This constituted an application of approximately 1.7 g of the sunscreen formulation applied to a total area of approximately 860 cm^2. This lotion contains 8% HS, 7.5% OMC, 6% OX, and 5% OS as active ingredients. Baseline blood and urine samples were collected prior to sunscreen application. Permeation of sunscreen into the skin, systemic absorption and urinary elimination were monitored for up to 48 h following application. At 30 min after application, a small area of the skin was wiped with Kleenex tissue and skin strip samples taken by application and removal of Scotch® crystal clear tape (3 cm × 1.9 cm). Tape stripping is a relatively non-invasive technique, which permits samples of stratum corneum (0.5–1 μm thickness) to be collected from the treated area. The tapes were applied to the treated areas by application of a consistent pressure generated by stroking the thumb 10 times along the tape. The stratum corneum was sequentially stripped up to 16 times and the 16 strips taken from each site were grouped into four groups for subsequent analysis of sunscreen content (group 1: strip 1; group 2: strips 2–6; group 3: strips 7–11; group 4: strips 12–16). This procedure was repeated on a separate site at 4 and 8 h. The stratum corneum was removed by 16 sequential strips, focussing on the upper layers of the stratum corneum and not affecting layers underneath the stratum

corneum. It has been found that on the flexor surface of the forearm about 30 tape strips are needed to strip off most of the horny layer [12]. Complete removal of the stratum corneum was not possible even after 30–40 strippings [13], and a certain barrier function in the tissue so treated remains [14,15]. Ohman and Vahlquist showed that after 100 tape strippings, the entire stratum corneum could be removed [16]. The stripping procedure was not normalized, since the inconsistent cohesion of the corneocyte layers means that reproducible amounts of SC (within and between subjects) cannot be removed [17]. The product was washed off the skin at 8 h post-application. Blood samples were taken from all subjects at pre-application baseline and at 1, 2, 4, 6, 8, and 24 h post-application. Urine output of all subjects over the 48 h post-application period was collected. All blood and tape strip samples were analyzed as described.

Sunscreen in the stratum corneum tape strip samples was extracted by a two-step procedure adapted from Potard et al. [18]. This involved overnight contact of the tape with isopropanol to destructure the polymeric glue, followed by dissolution of the polymeric glue and the hard polymeric tape support by acetonitrile. The solvent was then evaporated and the residue re-suspended in 1 ml methanol for analysis of sunscreen content by HPLC.

3. Results and discussion

3.1. Chromatography and resolution

HPLC chromatograms of the four sunscreen agents after sample preparation from an extract of 4% (w/v) BSA, plasma and tape strips are shown in Fig. 2. Many of the HPLC assays published are designed for product evaluation and determination of concentrations in cosmetic formulations [6–8,19]. The assay method previously published for evaluation of sunscreens in biological samples is useful but offers certain limitations in terms of sensitivity, especially with octylsalicylate [5]. Increased sensitivity is particularly important while measuring biological samples. Moreover, the method does not include the UV-B filter homosalate, which is present in many of the commercially available sunscreen formulations. Homosalate is particularly difficult to measure due to the low extinction coefficient, and the presence of two peaks corresponding to two isomeric forms. The two peaks due to homosalate are H1 and H2 (Fig. 2). H2 was used for calibration and quantitation. The present method, which includes homosalate, also provides increased sensitivity and resolution for many of the sunscreens measured compared to previous published methods. The procedure is relatively rapid with a run time of only approximately 10 min.

3.2. Linearity

Table 1 reports the results for calibration plot linearity. Excellent linearity was obtained over the range 0.1–5.0 μg/ml for the four sunscreen agents.

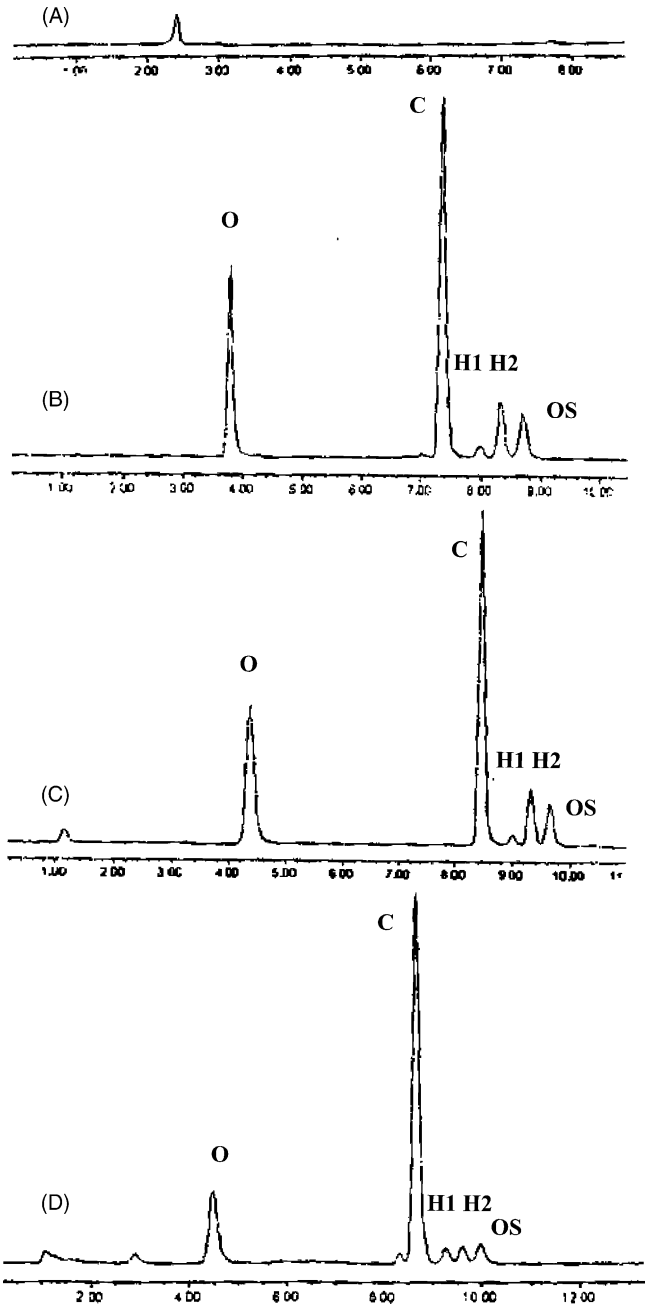

Fig. 2. Chromatograms of a blank of 4% (w/v) BSA (A), an extract from 4% (w/v) BSA in phosphate buffer (B), plasma (C) and skin strips (D). Peaks: (O) oxybenzone; (C) octylmethoxycinnamate; (H1 and H2) homosalate; (OS) octylsalicylate.

3.3. Assay precision

Calibration graphs were constructed by plotting the peak area versus concentration of standards injected. The best straight lines were determined using the method of least squares. To obtain a satisfactory UV response for all the analytes, each chemical was measured at its wavelength of maximum absorbance: oxybenzone at 289 nm, octylmethoxycinnamate at 310 nm, homosalate and

Table 1
Quantitative results for HPLC assay of sunscreens

	OX	OMC	OS	HS
Wavelength (nm)	289	310	237	237
Linear range (μg/ml)	0.1–5.0	0.1–5.0	0.1–5.0	0.1–5.0
Slope ($\times 10^6$)	3.53 ± 0.037	4.42 ± 0.20	2.01 ± 0.026	1.81 ± 0.026
Intercept ($\times 10^4$)	−(5.76 ± 2.1)	(2.15 ± 0.31)	−(3.78 ± 1.3)	−(3.47 ± 1.95)
Correlation coefficient	0.9999	0.9998	0.9998	0.9998
Minimum detection limit (ng)	0.8	0.3	2	2
Inter-day variation, mean ± S.D. (%CV)				
At 0.1 μg/ml	0.092 ± 0.002 (2.72)	0.1002 ± 0.004 (4.139)	0.101 ± 0.005 (5.439)	0.983 ± 0.005 (5.594)
At 5 μg/ml	4.976 ± 0.0304 (0.612)	5.01 ± 0.0357 (0.713)	4.99 ± 0.027 (0.557)	5.02 ± 0.037 (0.7457)
Intra-day variation, mean ± S.D. (%CV)				
At 0.1 μg/ml	0.095 ± 0.002 (2.47)	0.099 ± 0.005 (5.55)	0.105 ± 0.004 (4.15)	0.107 ± 0.005 (4.94)
At 5 μg/ml	4.985 ± 0.058 (1.16)	4.965 ± 0.146 (2.95)	4.947 ± 0.058 (1.176)	4.953 ± 0.048 (0.998)

R.S.D. of peak area ($n = 6$).

octylsalicylate at 237 nm. The intra- and inter-day variation for the four sunscreens was less than 3% at the upper end of the linear range and less than 6% at the lower end (as summarized in Table 1). There was no significant difference between day-to-day analysis (slopes evaluation, $P < 0.001$).

3.4. Minimum detectable limits

The lower limits of quantitation calculated as greater than 10 times the baseline noise in the assay were 2 ng (0.2 μg/ml) for oxybenzone, 1 ng (0.1 μg/ml) for octylmethoxycinnamate and 4 ng (0.4 μg/ml) for homosalate and octylsalicylate. The minimum detectable limits, calculated as greater than three times the baseline noise level in the assay, were 0.8 ng (0.08 μg/ml) for oxybenzone, 0.3 ng (0.03 μg/ml) for octylmethoxycinnamate, and 2 ng (0.2 μg/ml) for homosalate and octylsalicylate.

The limits of detection in a previously published assay by Jiang et al. were 0.1 ng (0.01 μg/ml) for oxybenzone, 1 ng (0.1 μg/ml) for octylmethoxycinnamate and 5 ng (0.5 μg/ml) for octylsalicylate [5]. Chisvert et al. reported detection limits of 1.7 μg/ml for oxybenzone, 2.2 μg/ml for octylmethoxycinnate, 2.3 μg/ml for homosalate and 1.5 μg/ml for octylsalicylate [6]. These are not directly comparable with the current data as the method for determination of minimum detection limits is different. Jiang et al. argued that lower sensitivity, particularly for octylsalicylate was because of compromise wavelength [5]. A similar wavelength was used by Chisvert et al. [6]. This has been resolved in our method, leading to increased sensitivity. In addition to the use of multiple wavelengths, gradient flow in our method favored better peak shape and separation. Therefore, this method would be more useful for measuring sunscreen agents, especially in biological samples.

3.5. Recovery study in human skin, plasma, urine and 4% (w/v) BSA in phosphate buffer

The recovery of the four sunscreen agents from plasma and 4% (w/v) BSA solution is summarized in Table 2. Recoveries were within the range of 90–104%. The coefficients of variation calculated from the six replicates were all less than 5%. Extraction of the sunscreens from the epidermal membranes and urine is also summarized in Table 2. Recoveries were within the range of 98–100 and 86–92%, respectively. Data for oxybenzone and its metabolites are summarized in Tables 2 and 3.

3.6. Preliminary volunteer study—absorption and distribution of sunscreens following topical application

This preliminary study demonstrates the application of the assay and extraction procedures developed. Sunscreens are regularly applied to large areas of the body and therefore it is essential to have an understanding of their safety.

Table 2
Recovery of sunscreens from human plasma, 4% (w/v) BSA in phosphate buffer and urine spiked with 0.5 or 5 μg/ml of each sunscreen

	OX	OMC	OS	HS
0.5 μg/ml plasma				
Recovery (%)	99.29	103.83	97.33	95.92
%CV	2.75	3.78	2.22	4.10
5 μg/ml plasma				
Recovery (%)	98.4	96.38	92.61	90.76
%CV	3.47	2.53	2.13	4.41
0.5 μg/ml of 4% (w/v) BSA				
Recovery (%)	102.1	99.47	102.31	97.95
%CV	2.56	1.90	2.22	3.28
5 μg/ml of 4% (w/v) BSA				
Recovery (%)	100.91	101.25	100.88	98.30
%CV	1.45	2.38	1.18	2.70
5 μg/ml urine				
Recovery (%)	90.03	89.22	86.82	92.25
%CV	3.05	4.45	4.32	3.22
Epidermal membranes				
Recovery (%)	98.84	99.20	98.49	99.55
%CV	2.53	1.82	3.07	3.20

Mean of six extractions.

Table 3
Recovery of oxybenzone and its metabolites from urine spiked with 5 μg/ml of each chemical

	OX	DHMB	DHB	THB
Recovery (%)	89.88	94.88	92.76	93.21
%CV	2.84	2.39	2.49	4.13

DHMB: 2,2′-dihydroxy-4-methoxybenzophenone; DHB: 2,4-dihydroxybenzophenone; THB: 2,3,4-trihydroxybenzophenone. Mean of six extractions.

In particular, it is necessary to quantify the skin penetration and distribution of sunscreen agents. Even if the degree of penetration is low, as the sunscreen product may be applied to a large surface area on a regular basis the total amount absorbed may be significant and the potential consequences should be considered. To date, a systematic investigation of sunscreen absorption and the influence of formulation has not been undertaken. In addition, as anatomical site has been shown to influence drug absorption, the difference in skin penetration after topical application to the arms and back was also considered. A significant amount of sunscreen penetrates the epidermal barrier (Fig. 3 and Table 4), a finding which is consistent with previously published in vitro and in vivo research [3]. Higher amounts of sunscreens were recovered from the upper layers of stratum corneum at 30 min post-application. At 4 and 8 h post-application, similar depth of penetration profiles were obtained but with overall lower sunscreen concentration (Table 4). There was no significant difference in the absorption of sunscreens

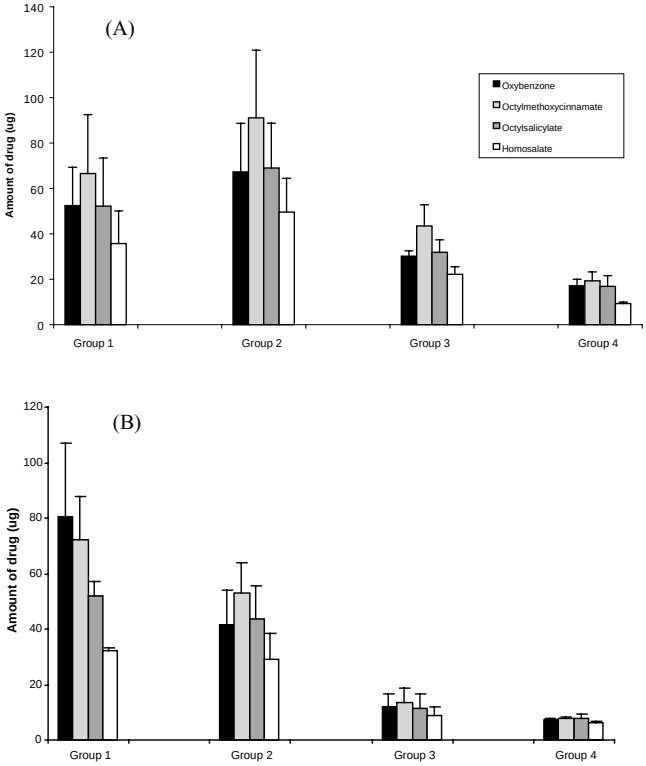

Fig. 3. Amount of sunscreen in the tape strips from the arms (A) and back (B) of volunteers after 30 min of application of the sunscreen formulation.

Table 4
Amount (μg per pool of strippings) of UV filters recovered from the tapes ($n = 3$)

Stripping group	After 30 min		After 4 h		After 8 h	
	Arm	Back	Arm	Back	Arm	Back
Group 1						
Oxy	52.39 ± 16.87	80.33 ± 26.44	**8.69 ± 3.61**	31.76 ± 6.49	19.78 ± 14.44	21.56 ± 4.10
OMC	66.59 ± 25.88	72.42 ± 15.22	**10.91 ± 2.76**	40.07 ± 5.18	25.67 ± 13.20	27.95 ± 4.62
OS	52.21 ± 21.12	51.925 ± 5.10	**9.88 ± 1.10**	32.20 ± 4.34	10.89 ± 3.39	24.44 ± 7.43
HS	35.71 ± 14.32	32.21 ± 1.24	**6.36 ± 1.82**	21.32 ± 7.25	13.28 ± 8.12	14.49 ± 4.06
Group 2						
Oxy	67.29 ± 21.37	41.37 ± 12.71	50.78 ± 32.60	29.42 ± 7.61	25.53 ± 16.59	16.09 ± 2.39
OMC	91.07 ± 29.75	52.74 ± 11.17	67.66 ± 41.45	35.93 ± 8.26	38.51 ± 24.53	21.12 ± 5.45
OS	69.01 ± 19.70	43.41 ± 11.95	54.21 ± 31.21	29.24 ± 4.69	26.93 ± 17.94	19.79 ± 9.15
HS	49.55 ± 14.86	29.24 ± 9.36	36.84 ± 21.36	19.6 ± 4.68	16.27 ± 12.53	11.9 ± 2.68
Group 3						
Oxy	**30.14 ± 2.32**	12.03 ± 4.81	16.49 ± 8.53	8.25 ± 3.60	23.24 ± 8.62	7.42 ± 1.22
OMC	**43.46 ± 9.30**	13.63 ± 5.20	22.59 ± 11.07	11.17 ± 3.35	12.30 ± 6.36	10.05 ± 2.33
OS	**31.89 ± 5.49**	11.67 ± 5.10	18.905 ± 9.71	10.12 ± 3.92	10.41 ± 4.61	9.23 ± 3.09
HS	22.12 ± 3.39	8.66 ± 3.39	13.67 ± 7.71	6.09 ± 2.02	6.35 ± 4.26	5.43 ± 0.64
Group 4						
Oxy	**17.12 ± 2.88**	7.18 ± 0.67	11.98 ± 6.05	4.86 ± 0.64	6.29 ± 1.65	4.58 ± 2.10
OMC	**19.29 ± 3.94**	8.02 ± 0.54	12.61 ± 2.54	7.27 ± 3.53	10 ± 1.91	6.77 ± 4.95
OS	16.86 ± 4.71	7.9 ± 1.42	10.17 ± 1.68	7.51 ± 5.7	7.5 ± 1.56	6.65 ± 5.63
HS	9.27 ± 0.63	6.00 ± 0.84	7.43 ± 2.04	4.14 ± 1.94	4.23 ± 1.01	3.63 ± 2.56

Oxy: oxybenzone; OMC: octylmethoxycinnamate; OS: octylsalicylate; HS: homosalate. Group 1: strip 1; group 2: strips 2–6; group 3: strips 7–11; group 4: strips 12–16. Bold numbers are significantly different ($P < 0.05$). Values for arms are compared with back at a particular time.

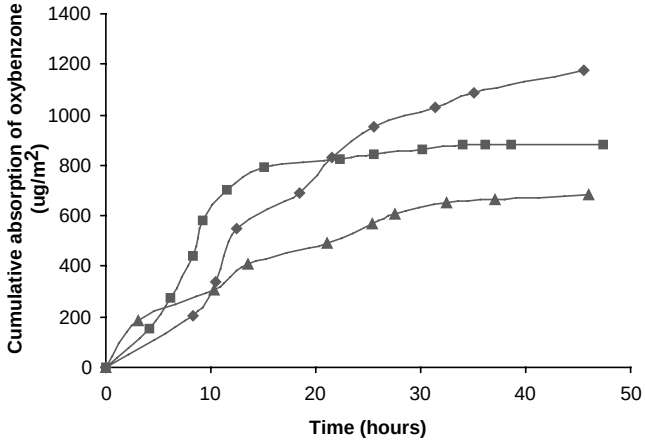

Fig. 4. Systemic absorption of oxybenzone following topical application of a commercial sunscreen product to three healthy human volunteers; absorption was determined from urinary excretion of oxybenzone and its metabolite.

into the skin after application to the arms and back, though slight differences were observed (shown in bold in Table 4). This is consistent with previous reports of similar absorption kinetics through the arms and back after topical application of other drugs [20,21]. In addition, the amount quantified in the first strip (group 1) seems to predict which components will have better substantivity for the stratum corneum. From Fig. 3, it appears that oxybenzone, octylmethoxycinnamate and octylsalicylate have more affinity towards the stratum corneum when compared to homosalate.

Systemic absorption of oxybenzone was confirmed through detection of oxybenzone in the blood/plasma and in the urine. The plasma was only measured for the four sunscreens, excluding their metabolites. Small amounts of oxybenzone (<130 ng/ml) were observed in the plasma, whereas other sunscreens were below the limits of detection. Up to approximately 1% of the applied dose of oxybenzone and its metabolites were detected in the urine (Fig. 4). The major metabolite was 2,4-dihydroxybenzophenone (DHB), whereas 2,3,4-trihydroxybenzophenone (THB) was detected only in trace amounts. The low levels of oxybenzone in the blood may be due to rapid metabolism and distribution, as has been demonstrated previously in rats [22]. Okereke et al. further reported that oxybenzone and its metabolites were found in liver, kidney, spleen, heart and even testes. Since the area of application of sunscreen in our study was approximately 864 cm^2, which is almost half the area that one could apply in beach sunbathing situation, the total amount of systemic absorption of oxybenzone could be higher in practice. In addition, tissue and systemic levels of sunscreens may be greater in young children who have less well-developed processes of elimination, and a larger surface area per body weight ratio than adults. Sunscreens are recommended to be applied frequently throughout the day, therefore the amount used in practice is likely to exceed the application amount of 2 mg/cm^2, also promoting penetration.

This assay provides an efficient means of quantifying the most common sunscreens in a range of biological matrices relevant to both in vitro and in vivo assessment of skin penetration. As such, it will facilitate the development of novel sunscreen products with high SPF and substantivity (skin retention) but also minimal absorption to deep tissues or the systemic circulation.

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