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Analysis of octyl methoxycinnamate in sunscreen products by a validated UV-spectrophotometric method

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Synopsis

An inexpensive, rapid method for the determination of octyl methoxycinnamate (OMC) in sunscreen products using ultraviolet-spectrophotometry has been developed and validated according to International Council for Harmonisation and Association of Official Analytical Chemists guidelines. Methanol was the optimal solvent used with a linearity range of 4–12 µg/ml (r = 0.999) being obtainable. The accuracy of the method is highlighted by the % recovery (98.23–98.50) and relative standard deviation (%RSD, 0.12), and it is widely applicable to prototype products composed of oil in water, and water in oil emulsions. Mineral oils containing low, intermediate, and high OMC levels (1%, 4%, and 7.5%) gave recovery percentages of 99.76–100.76 with %RSD of 0.02–0.28. In addition, this method is repeatable and affords a high degree of precision (%RSD = 0.12 and 0) with 96.08–99.27% recovery. The method is suitable for quality assurance of suncare product formulations, and could be applicable to product development and validation.

INTRODUCTION

Application of sunscreen products is widely recommended to protect against the harmful effects of ultraviolet (UV) radiation on skin (1), such as erythema, edema, and hypopigmentation, which alters its aesthetics, and accumulative damage which can result in melanoma (2). The UV filtering agents in sunscreens are commonly classified as organic or inorganic, in relation to their skin protection mechanism. The most commonly used UV filter is octyl methoxycinnamate (OMC; also listed as ethylhexyl *p*-methoxy cinnamate or octinoxate). Exposure of OMC to solar radiation generates reactive chemical species including free radicals. If OMC is present in sunscreens, there is a potential for accumulation of free radicals in tissue, giving rise to adverse effects (3). Accordingly, the maximum allowable concentrations of OMC in sun protection products are 10% and 7.5%, as stated in European Union and U.S. Food and Drug Administration regulations, respectively (4).

The presence of sunscreen agents in topical products is necessary to ensure their skin damage protection efficacy, although such formulations must also be stable (5) and meet

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quality assurance standards. Suncare actives (including OMC) are usually analyzed by techniques such as high-performance liquid chromatography (HPLC) (5–8), which often requires high levels of technical expertise, time (method development), and investment in advanced equipment, resulting in high cost per sample analyzed. Therefore, development of a simple, rapid, precise, accurate, and inexpensive technique which is practically feasible (5,9) would be of profound benefit to the pharmaceutical industry and preferred to the currently used routine practices in product formulation (10). Accordingly, this report highlights a simple, cost-effective method based on UV-spectrophotometry for the analysis of OMC in sunscreen products. The method has been validated in accordance with the International Council for Harmonisation (ICH) and Association of Official Analytical Chemists (AOAC) guidelines (11,12), with validated repeatability in three types of dosage forms: oil in water (O/W) and water in oil (W/O) emulsions, and mineral oil. The results indicate that this method should be widely applicable, and feasible for incorporation into suncare product testing protocols.

MATERIALS AND METHODS

Those cosmetic formulations were of cosmetic grade, whereas those of analytical practices were of analytical grade.

MATERIALS AND INSTRUMENTS

OMC (BASF, Duesseldorf, Germany) concentrated 1%, 4%, and 7.5% in sunscreen emulsion (O/W) consisted of isopropyl myristate (Kik.K Oleo, Selangor, Malaysia), isopropanol (Top solvent, Bangkok, Thailand), stearic acid (NOF, New York, NY), cetyl alcohol (Godrej industries, Mumbai, India), triethanolamine (Hangzhou, Zhejiang, China), carbopol 934 (Lubrizol, Wickliffe, Ohio), and deionized water. Oil phases were melted and mixed at 70°C before OMC addition, followed by the addition of water phase containing carbopol and triethanolamine to give homogeneous cream. W/O emulsion was formulated with petrolatum (Paraffin oil, India), emulsifying wax (Anglo-chemical, Free State, South Africa), calcium stearate (Peter Greven, Bad Münstereifel, Germany), caprylic/capric triglyceride (BASF), jojoba oil (Desert whale, Tucson, AZ), and microcrystalline wax (Hong Huat, Bangkok, Thailand) that were melted together at 80°C, followed by additions of deionized water and OMC. In addition, OMC was separately dispersed in mineral oil (Kesoon Fine Chemical, Maoming, China). The proportions of each ingredient of the prepared formulations were shown in Table I. A UV-visible spectrometer (UV mini 1240; Shimadzu, Tokyo, Japan) was used to record the absorbance. The product was sun protection factor (SPF) monitored by SPF analyzer (Optometrics LLC/SPF-290F, Miami, FL; 13).

VALIDATION OF OMC ANALYSIS METHOD

Specificity. OMC (0.05 g) was weighted (BP 2215; Sartorious, Göttingen, Germany) into a volumetric flask and adjusted with acetonitrile, ethanol, and methanol (Merck, Darmstadt, Germany) to a final volume of 50 ml individually. The solution (5 ml) was

Table I
Ingredients of O/W and W/O Emulsions

Emulsion	Ingredient	% (w/w)
O/W	Isopropyl myristate	12.0
	Isopropanol	3.0
	OMC	1.0 or 4.0 or 7.5
	Stearic acid	1.0
	Cetyl alcohol	0.5
	Triethanolamine	1.0
	Carbopol 934	0.2
	Deionized water	qts. to 100
W/O	Petrolatum	17.0
	Emulsifying wax	4.0
	Calcium stearate	2.0
	OMC	1.0 or 4.0 or 7.5
	Caprylic/capric triglyceride	10.0
	Jojoba	10.0
	Microcrystalline wax	6.0
	Deionized water	qts. to 100

transferred into another volumetric flask (50 ml), and diluted into 5 µg/ml with the different solvents. An absorbance pattern of each solution was recorded during 200–400 nm. The best solvent affording maximized absorption without the presence of interference effects was obtained. The stock OMC solution (0.8 mg/ml) was prepared into serial dilutions (4, 8, and 12 µg/ml), which were further validated on linearity, range, accuracy, and precision of the analysis.

Linearity, range, accuracy, and precision. The above mentioned validated parameters were assessed at five different concentrations (4, 6, 8, 10, and 12 µg/ml), and the absorbance was recorded at 310 nm. The linearity was determined with a determination correlation (r) of more than 0.995, in addition to a recovery (%) calculation as well as a relative standard deviation (%RSD). All of the experiments were performed six times.

VALIDATION OF OMC SUNSCREEN ANALYSIS METHOD

Specificity. The cosmetic bases (8 g) were weighted into volumetric flasks (100 ml), separately, prior to the addition of methanol to achieve the final volume. The solution was filtrated through Whatman no. 5 filter paper. The filtered solution (5 ml) was pipetted into 50 ml volumetric flask and diluted into 5 µg/ml. The absorbance was determined as described in the specificity of OMC analysis.

Accuracy and precision. The cosmetic bases (8 g of 1% OMC, 2 g of 4% OMC, and 1.07 g of 7.5% OMC) were weighted into six volumetric flasks (100 ml). The stock OMC solution (0.8 mg/ml, 10 ml) in each flask was adjusted to 100 ml with methanol and mixed thoroughly. The solution was filtered and diluted into 5 µg/ml. The absorbance determination was done at 310 nm as mentioned earlier. The procedure was conducted in six replicates.

Repeatability of the method. OMC sunscreens in O/W and W/O emulsions, and mineral oil at different concentrations were weighted differently into the 100 ml volumetric flask

similar to the accuracy validation described previously. The absorbance was recorded and the experiment was repeated for five more times.

Repeatability of the system. The OMC preparations were weighted (8 g, each) and diluted into 5 μg/ml as above. The absorbance was determined at 310 nm. The practice was done in six replicates.

RESULTS AND DISCUSSION

VALIDATION OF OMC ANALYSIS METHOD

The proposed UV-spectrophotometric determination of OMC was validated in accordance with ICH and AOAC guidelines (11,12). The specificity of OMC determination was evaluated in different solvents: acetonitrile, ethanol, and methanol. The working wavelength (λ_{max}) for OMC determination was found to be 310 nm, resulting in maximum absorbance values, with methanol being the best solvent affording maximized absorption (≤1) for OMC (4, 8, and 12 μg) without the presence of interference effects. This specificity profile was adopted for further method validation.

Linearity and range. The validated parameters were assessed at five different concentrations (11,12) of OMC; 4, 6, 8, 10, and 12 μg/ml (Table II). This coefficient of determination for this range was 0.999 ($y = 0.086x - 0.004$), which being greater than 0.995 highlights the precision of the method with acceptable linearity (11,12), comparable to that of OMC analysis by HPLC ($r = 0.999$) at a higher detection limit (10–40 μg/ml; 6).

Accuracy and precision. The accuracy of the method is highlighted by the recovery (%) of OMC obtained after six analyses. Repeatability of the protocol was demonstrated by recoveries of 98.23–98.56% as shown in Table III, with these falling within the AOAC set range (12). Method precision was calculated in terms of %RSD, with a %RSD of less than 1 indicating a high level of precision (11). Furthermore, this method exhibited better reproducibility for OMC determination than HPLC (%RSD = 0.59) or micellar electrokinetic capillary chromatography (MECC; %RSD = 1.89) as applied over a wider linear range (0.02–20 μg/ml and 0.25–50 μg/ml; 6).

VALIDATION OF OMC ANALYSIS METHOD IN SUNSCREEN PRODUCTS

Sunscreen products containing OMC (1%, 4%, and 7.5%) in three different dosage forms: O/W and W/O emulsions, and mineral oil were SPF determined (Table IV) to

Table II
Linearity of OMC Analysis

OMC (μg/ml)	Absorbance
3.9968 ± 0.0070	0.3434 ± 0.0010
6.0153 ± 0.0030	0.5191 ± 0.0011
8.0377 ± 0.0014	0.6765 ± 0.0008
9.8625 ± 0.0005	0.8528 ± 0.0015
11.8290 ± 0.0046	1.0196 ± 0.0008

Table III
Accuracy and Precision of OMC Analysis

				%RSD	
Added amount (μg/ml)	Found amount (μg/ml)	Absorbance	Recovery (%)	The method	The system
8.0379	7.8953	0.675	98.23	0.12	0
8.0383	7.9070	0.676	98.37		
8.0392	7.9070	0.676	98.36		
8.0382	7.9186	0.677	98.51		
8.0380	7.9186	0.677	98.51		
8.0345	7.9186	0.677	98.56		

monitor their sun protection efficacies. Obtained UVB absorption efficacies of 50–87% (13) were based on the presence of OMC as a single UV filter, and thereafter all sunscreen products were further analyzed for OMC content.

Repeatability of the method. The absorbance values for cosmetic bases used in the SPF determinations above were recorded at wavelengths between 200 and 400 nm, with the baseline for each having a smooth pattern and showing no interference over the analytical range. The base was weighted (8 g) for 8 mg/ml OMC that was calculated from 1% OMC in the product. The analytical method is, therefore, specific for OMC determination in these prototype sunscreens. The accuracy of OMC levels in these sunscreens was further validated by weighing, conducted on the basis of OMC content at different amounts as described in the Materials and Method section. Recovery values (%) were in the range of 99.76–100.76%, with %RSD being less than 1 as shown in Table IV. Therefore, this analytical method provides an accurate determination of OMC on this basis (11). Interestingly, greater recoveries and lower RSD were obtained than in analysis of OMC creams by HPLC (95.6–103.5%, 1.0–2.3%; 8), including after extraction using supercritical fluid (99.55–103.31%; 7).

Repeatability of the system. OMC sunscreens were subjected to repeat analysis (six times) using the procedure described above. The reproducibility (Table V) was confirmed by the recovery (%) range (97.23–99.27), and % RSD (0.08–0.50).

Table IV
Repeatability of the Analytical Method

Preparation	OMC (%)	Recovery ± RSD (%)
O/W emulsion	1	99.98 ± 0.06
	4	99.76 ± 0.08
	7.5	100.04 ± 0.23
W/O emulsion	1	100.02 ± 0.12
	4	100.76 ± 0.02
	7.5	99.98 ± 0.09
Mineral oil	1	100.04 ± 0.28
	4	99.93 ± 0.10
	7.5	100.04 ± 0.10

Table V
Repeatability of the Analytical System

Preparation	OMC (%)	SPF	Recovery ± RSD (%)
O/W emulsion	1	2.83 ± 0.11	96.08 ± 0.14
	4	5.12 ± 0.79	98.50 ± 0.08
	7.5	8.27 ± 0.59	97.23 ± 0.29
W/O emulsion	1	2.18 ± 0.43	99.06 ± 0.10
	4	6.30 ± 0.48	97.56 ± 0.16
	7.5	8.28 ± 0.90	99.11 ± 0.17
Mineral oil	1	2.50 ± 0.22	98.25 ± 0.50
	4	6.29 ± 0.62	98.01 ± 0.10
	7.5	8.65 ± 0.76	99.27 ± 0.19

CONCLUSIONS

Determination of OMC in sunscreens was achieved using a UV-spectrophotometric method validated according to ICH and AOAC guidelines (11,12). Methanol was found to be the optimal solvent, with the method showing an analytical linearity range between 4 and 12 µg/ml with a determination coefficient of 0.999. Furthermore, the method was applicable to emulsions containing 1%, 4%, and 7.5% of OMC, including those in mineral oil. Thus, this method is an inexpensive, simple, rapid, accurate, and precise way to determine the OMC content in sunscreen products (5), and could be a viable alternative to HPLC and MECC methods (2,5,7,8). However, this validated method is only suitable for sunscreen products comprising solely OMC as the UV filter, without some type of chromatographic separation, any other chemical species with an absorbance in the range of OMC could cause interference and might lead to erroneous results. The challenge is to expand the scope of this method for the detection of different suncare actives and for use in other formulations to enhance its potential quality assurance applications in the pharmaceutical industry.

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REFERENCES

(1) E. Gilbert, F. Pirot, V. Bertholle, L. Roussel, F. Falson, and K. Padois, Commonly used UV filter toxicity on biological functions: Review of last decade studies, *Int. J. Cosmet. Sci.,* **35,** 208–209 (2013).
(2) P. Kullavanijaya and H. W. Lim, Photoprotection, *J. Am. Acad. Dermatol.,* **52,** 937–958 (2005).
(3) K. Jung, M. Seifert, Th. Herrling, and J. Fuchs, UV-generated free radicals (FR) in skin: Their prevention by sunscreens and their induction by self-tanning agents, *Spectrochim. Acta A.,* **69,** 1423–1428 (2008).
(4) A. O. Barel, M. Pay, and H. I. Maibach, *Handbook of Cosmetic Science and Technology, 3rd Ed*. (Informa Healthcare, New York, NY, 2009).
(5) J. Cheng, Y.-L. Li, R. L. Roberts, and G. Walker, Analysis of 2-ethylhexyl-*p* methoxycinnamate in sunscreen products by HPLC and Raman spectroscopy, *Talanta,* **44,** 1807–1813 (1997).
(6) Y. Shih and F.-C. Cheng, Determination of sunscreen agents in cosmetic products using microwave-assisted extraction and liquid chromatography, *J. Chromatogr. A.,* **876,** 243–246 (2000).

(7) S.-P. Wang and W.-J. Chen, Determination of *p*-aminobenzoates and cinnamate in cosmetic matrix by supercritical fluid extraction and micellar electrokinetic capillary chromatography, *Anal. Chim. Acta.,* **416,** 157–167 (2000).
(8) S. Simeoni, R. Tursilli, A. Bianchi, and S. Scalia, Assay of common sunscreen agents in suncare products by high-performance liquid chromatography on a cyanopropyl-bonded silica column, *J. Pharm. Biomed. Anal.,* **28,** 250–255 (2005).
(9) S. K. Motwani, S. Chopra, F. J. Ahmad, and R. K. Khar, Validated spectrophotometric methods for the estimation of moxifloxacin in bulk and pharmaceutical formulations, *Spectrochim. Acta A.,* **68,** 250–256 (2007).
(10) P. Charoennit and N. Lourith, Validated UV-spectrophotometric method for the evaluation of the efficacy of makeup remover, *Int. J. Cosmet. Sci.,* **34,** 190–192 (2012).
(11) The European Agency for the Evaluation of Medicinal Products, *ICH Topic Q2B Note for Guideline on Validation of Analytical Procedure: Methodology* (The European Agency for the Evaluation of Medicinal Products, London, UK, 1996).
(12) Association of Official Analytical Chemists International, Rockville, MD (AOAC guideline for single laboratory validation of chemical methods for dietary supplement and botanicals 2012).
(13) N. Lourith and M. Kanlayavattanakul, Appraisal of Thai glutinous rice husk for health promotion products, *J. Cereal Sci.,* **57,** 343–347 (2013).