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RESEARCH ARTICLE

A validated HPLC method for simultaneous estimation of Melatonin and Octyl Methoxycinnamate in combined pharmaceutical applications

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ABSTRACT

A simple, fast and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Melatonin and Octyl Methoxycinnamate. Melatonin has become an attractive substrate in sunscreen formulations because of its high antioxidant and photo-protection properties. Octyl methoxycinnamate is one of the chemical UV filter that can be found most of the sunscreen formulations up to 7.5 % according Food and Drug Administration. The aim of the present study was to develop and validate a High-Performance Liquid Chromatography method for the determination of Melatonin and Octyl Methoxycinnamate in combined pharmaceutical or cosmetic applications. As a model of combined pharmaceutical applications, a microemulsion consisting of Melatonin and Octyl Methoxycinnamate was also prepared and characterized in terms of droplet size, pH and viscosity. The separation was performed with a Waters XTerra RP C18 (5 µm, 4.6 x 150 mm). All HPLC assays were performed with 10 µl injection volume, mobile phase consisting of acetonitrile and water, using gradient elution starting at 20% and ending at 90% of acetonitrile with a flow rate of 1.5 ml min^{-1}.The eluent was monitored with UV detection at 222 nm for Melatonin and 306 nm for Octyl Methoxycinnamate. The method was validated according to ICH guidelines. Validation parameters were specificity, accuracy, precision (repeatability and reproducibility), linearity, limit of detection (LOD) and limit of quantification (LOQ). Analytical method development results indicated that the LOD values were 0.132 and 0.049 µg/ml; LOQ values were 0.4 and 0.15 µg/ml and assay exhibited a linear range of 0.5- 60 µg/ml for Melatonin and Octyl Methoxycinnamate, respectively.

Key words : Melatonin (PubChem CID: 896); Octyl methoxycinnamate (PubChem CID: 5355130) ; RP-HPLC method development.

1. Introduction

Melatonin (MEL) is a well-known neuroendocrine mediator produced by the pineal gland that was discovered in 1960s [1]. Melatonin is involved in numerous biological functions including circadian rhythm, sleep, the stress response, aging, and immunity. Beside those, melatonin is also synthesized in skin activated by a local melatoninergic system in protection against ultraviolet radiation (UVR) induced damages. Therefore, combination of endogenous and topically applied exogenous melatonin makes this molecule attractive as a potent antioxidant for sunscreen formulations [2-4].

Sunscreen formulations are composed of different chemicals and UV filters are the most essential ingredients among them. Octyl methoxycinnamate (OMC) is a widely used UV-B filter which absorbs radiation in the 290–320 nm region of the UV spectrum. According to Food and Drug Administration (FDA) regulations, sunscreen products can contain OMC up to 7.5 % [5].

There are different factors affecting the consumers' choice about the sunscreen products. In recent years, transparent

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dispersions have been used as popular sunscreens. Microemulsions are thermodynamically stable colloidal systems consisting of oil and water phases which are stabilized by surfactants and co-surfactants [6]. Their use as topical delivery systems derives from their multiple advantages compared to other dermatological formulations, such as ease of preparation, thermodynamic stability and penetration-enhancing properties [7]. Microemulsions are optically isotropic, transparent or slightly opalescent drug delivery systems with low viscosity. These features ensure the production of transparent, nonsticky and easily spreadable formulations [8].

A number of different determination methods have been developed for MEL and OMC individually [9, 10]. There is no any analytical method that has been reported yet for determination of these drugs together. Therefore, the present research work aims to develop a simple, sensitive, accurate and reproducible method for simultaneous estimation MEL and OMC in combined pharmaceutical applications by a single Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method. A microemulsion formulation consisting of MEL and OMC was used as a combined model system of the two active pharmaceutical ingredient (API). Microemulsion was prepared using pseudoternary phase diagrams and characterized in terms of droplet size, pH and viscosity.

2 Materials and Methods

2.1 Materials

Melatonin was purchased from BioShop (Burlington, Ontario, Canada). OMC was a kind gift from Mikrogen (Istanbul, Turkey). Tween 80 was purchased from Merck Transcutol P was obtained from Gattefossé (Lyon, France). All solvents were HPLC grade and purchased from Merck (Darmstadt, Germany). Highly purified water was obtained through ultrapure water system (Model-Arium 611) of Sartorius AG (Goettingen, Germany).

2.2 Preparation and Characterization of Microemulsions

Nine microemulsions which were composed of isopropyl myristate (IPM) (oil phase) and Tween80:Trascutol P (surfactant:co-surfactant) with different (w/w) ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) were prepared with a total weight of 5 g in a glass beaker. Surfactant and co-surfactant ratio was kept 2:1 at all times. Deionized water was added drop wise to these microemulsions with continuous stirring using a magnetic stirrer until the it begins to be slightly turbid and then turns over clear with continuous rotation and total water (aqueous phase) amounts were recorded. Pseudoternary phase diagrams were constructed using Triangle Phase Diagram software. Samples exhibiting a transparent and homogeneous state were assigned to a microemulsion region in the phase diagram. After the microemulsion regions in the phase diagram were identified, typical microemulsion vehicles were selected and prepared with addition of 1 % MEL and 5 % OMC (w/w). Both MEL and OMC have lipophilic characters, so they were dissolved in oil phase. The molecular structures of the APIs are shown in Figure 1.

The average droplet size and polydispersity index of microemulsions were measured at 25 ± 2 °C by photon correlation spectroscopy (Nano ZS, Malvern Instruments,

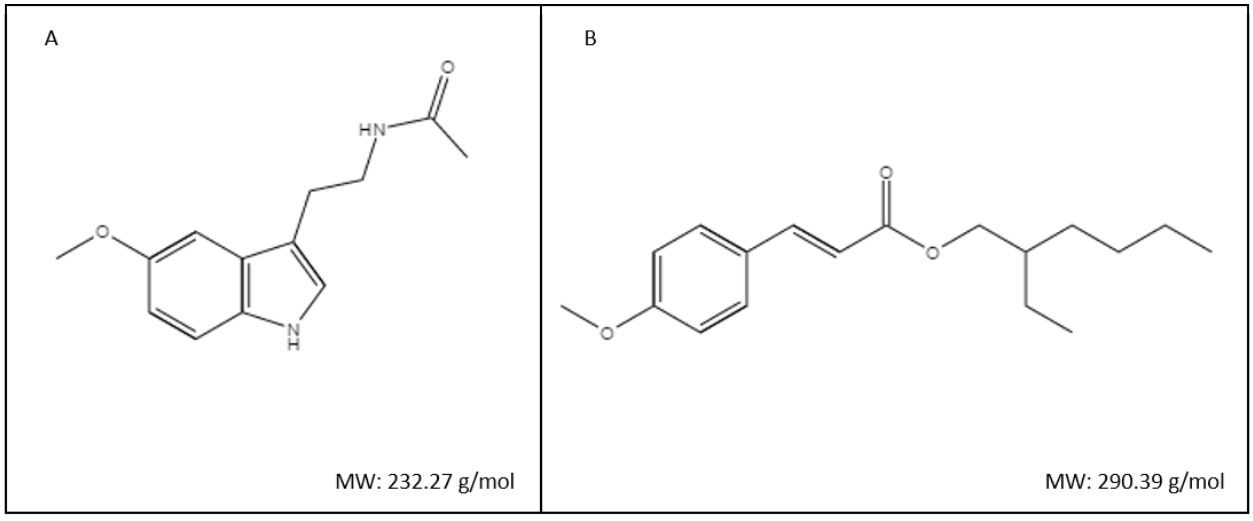

Figure 1. Chemical structures of (A) melatonin and (B) octyl methoxycinnamate.

UK). The pH values were detected at 25 ± 2 °C using a digital pH-meter (HI 221 – Mauritius). The viscosities of microemulsions were measured at 25 ± 2 °C using a viscometer (AND Vibro Viscometer SV10).

2.3 Instrumentation and Chromatographic Conditions

An Agilent 1100 HPLC system (Wilmington, DE, USA) equipped with a solvent degasser, quaternary pump, autosampler, column oven and a diode array detector was used in the study. Agilent ChemStation software was used for instrument operation control and data collection. Waters XTerra RP C18 (5 µm, 4.6 x 150 mm) column was used for separation. All HPLC assays were performed with 10 µl injection volume, mobile phase consisting of acetonitrile and water, using gradient elution starting at 20 % and ending at 90 % of acetonitrile with a flow rate of 1.5 ml min^{-1}. The details of gradient elution can be found at Table 1. The column oven was conditioned at 25 °C and analysis time was 15 minutes including re-equilibration time. λmax values were determined by scanning with DAD detector between 200-400 nm. Prior to the injection, column was equilibrated until the UV signal and back pressure were stabilized with the mobile phase flowing through the system.

Table 1. Gradient conditions of the proposed HPLC method

T (min)	A % (ACN)	B % (Water)
6.50	20	80
6.51	90	10
11.00	90	10
11.10	20	80

2.4 Analytical Method Validation

The described method was validated according to ICH guidelines [12] with respect to specificity, accuracy, precision (repeatability and reproducibility), linearity, limit of detection and limit of quantification.

2.4.1 Preparation of Standard Solutions

Stock solutions of MEL and OMC were prepared by dissolving 10 mg of each API in PBS:ethanol (1:1) (v/v) mixture in a 10 ml volumetric flask. Standard calibration solutions of MEL and OMC having concentrations in the range of 0.5-60 µg/ml were prepared by diluting stock solutions with PBS:ethanol (1:1) (v/v) mixture.

2.4.2 Specificity

Specificity of an analytical method shows the detection ability of the desired component(s) in the presence of other components (i.e. excipients) that may be expected to be present in the sample. In order to determine the specificity of the analytical method, dilution solution, PBS:ethanol (1:1) (v/v) and the placebo solution that contains the microemulsion formulation without the APIs were injected into the chromatographic system.

2.4.3 Accuracy

The accuracy of the method was determined by recovery studies which help the comparison of the experimental amount and the theoretical amount. The recovery studies were evaluated in triplicate using three concentrations, 40, 50 and 60 µg/ml.

2.4.4 Precision

The precision of method was verified by obtaining repeatability, intermediate precision and reproducibility parameters. Repeatability was checked by injecting six individual sample preparations of MEL and OMC at 50 µg/ml concentration. In order to determine the intermediate precision, six individual samples at a concentration of 50 µg/ml was prepared and analyzed by two analysts. Reproducibility of the system was determined by preparation of six different samples from the same stock solution at the same concentration of 50 µg/ml. Standard deviation (SD) and relative standard deviation (RSD) of the area for each API were calculated and evaluated.

2.4.5 Linearity

The linearity between peak area and concentration was analyzed using calibration curve obtained from six standard solutions of MEL and OMC at 60, 40, 20, 10, 5 and 1 µg/ml concentrations in triplicate. The calibration curves were plotted with the responses of peak area versus concentration of analyte and fitted using least squares linear regression.

2.4.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ value determinations were based on the standard deviation of the response and the slope. Following equations (1) and (2), where σ is the standard deviation of

the response and S is the slope of the calibration curve, were used to calculate the LOD and LOQ based on the calibration curve.

$$LOD = 3.3\ \sigma/S \quad (1)$$

$$LOQ = 10\ \sigma/S \quad (2)$$

2.5 Ex-vivo Permeation Study of the MEL-OMC Microemulsion Formulation

Ex-vivo permeation studies were performed on Wistar-albino rat's abdominal skin samples mounted on vertical glass Franz-type diffusion cells with an effective diffusion area of 0.384 cm^2. The skin samples were excised immediately after the sacrificing the animas used for another study, and were purified from the surrounding tissue and hair on top. In the receptor phase, PBS:Ethanol (1:1) pH 7.4 was used as the medium and constantly stirred with a magnetic stirrer at 600 rpm. The sink conditions were taken into consideration during the experiment. The system was kept at 37°C±0.2 for 24 hours. At the end, skin surface was cleaned by removing the excess formulation. Skin samples were cut into small pieces and accumulated APIs in the skin was extracted with the aid of a horizontal shaker in 5 ml of methanol for 24 hours. The amount of MEL and OMC in samples were quantified with the HPLC method.

2.6 Stability Studies of the MEL-OMC Microemulsion Formulation

Stability studies were performed in stability cabinets at 40 °C with 75 % relative humidity (RH), 25 °C with 60 % RH and at 4 °C for up to 3 months in terms of conductivity, droplet size, viscosity, pH and MEL and OMC concentration. The samples were analyzed by the newly developed HPLC.

3 Results and Discussion

3.1 Preparation and Characterization of Microemulsions

Pseudoternary phase diagram was constructed using IPM as oil, Tween 80 as surfactant and Transcutol P as co-surfactant. Figure 2 represents a large microemulsion area of 696.82 which was obtained with 2:1 surfactant and co-surfactant

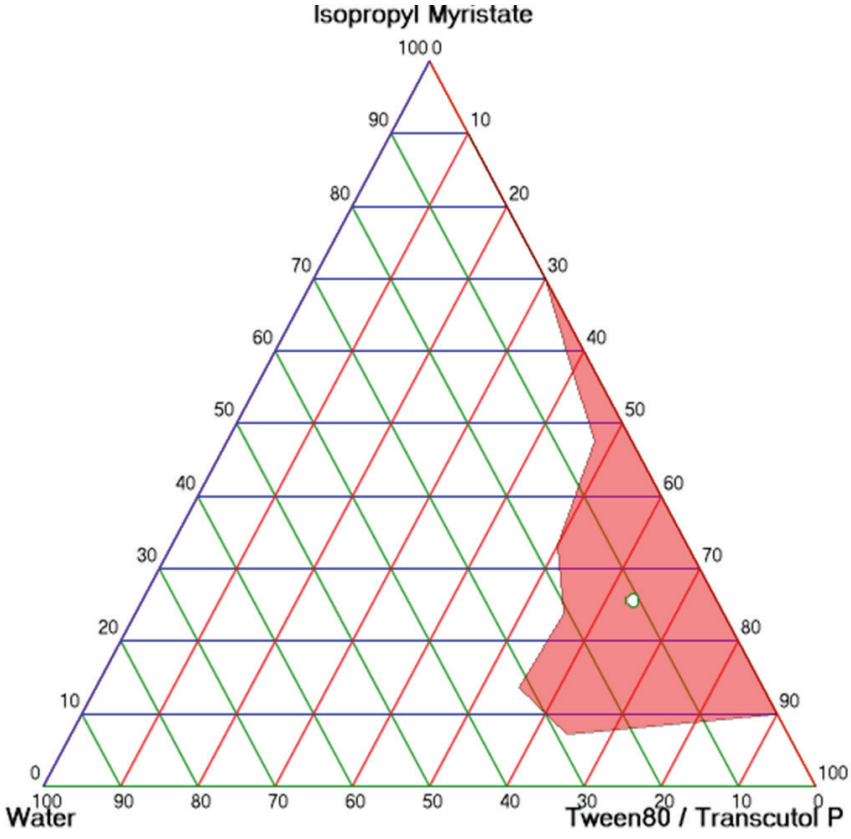

Figure 2. Pseudoternary phase diagram of the microemulsion formulation.

mixture. The formulation consisting of 11 % oil phase and 63 % surfactant and co-surfactant mixture was selected from the microemulsion region and 1 % MEL and 5 % OMC was added to the oil phase then the drug loaded formulation was characterized in terms of droplet size, polydispersity index, pH and viscosity. The drug loaded formulation has a droplet size of 193 ± 1.41 nm with a polydispersity index of 0.471 ± 0.032. The acidity of the skin ranges from pH 4 to 6 and pH levels ranging from 5-8 are acceptable for topical applications [13]. The pH of the formulation was found 6.35 ± 0.02 indicating that the formulation is suitable for topical application. The formulation showed a low viscosity and measured as 360 ± 0.57 cP at 25 ± 2°C.

3.2 Analytical Method Validation

The summary of the validation parameters for the proposed method is presented in Table 2. Theoretical plate number is a measure of column efficiency. The efficiency of the method is expressed by the number of theoretical plates (N), which assumed the value of 5490 and 111863 for MEL and OMC, respectively. These values are in agreement with FDA's recommended parameter of N > 2000 [14]. The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak. The symmetry of the peak is established by symmetry factor. As peak symmetry moves away from values of 1, integration, and hence precision, become less reliable (USP 37). Symmetry factors were calculated by The HP ChemStation and determined as 0.91 and 0.9 for MEL and OMC, respectively.

Table 2. Summary of validation parameters for the proposed method.

Parameters	MEL	OMC
λmax (nm)	222	306
Retention Time (min)	5.6	9.6
Theoretical plates	5490	111863
Tailing factor	0.91	0.9
Linearity range (µg/mL)	0.5-60	0.5-60
Correlation coefficient (r)	0.9995	0.9991
LOD (µg/mL)	0.132	0.049
LOQ (µg/mL)	0.4	0.15

The UV spectrum of MEL and OMC is presented in Figure 3. The UV spectrum of APIs in mobile phase was scanned in the region between 200 to 400 nm. The λmax values were determined at 222 nm and 306 nm for MEL and OMC, respectively. MEL showed a retention time at 5.6 min whereas OMC peak was seen at 9.6 min resulting that APIs were separated successfully.

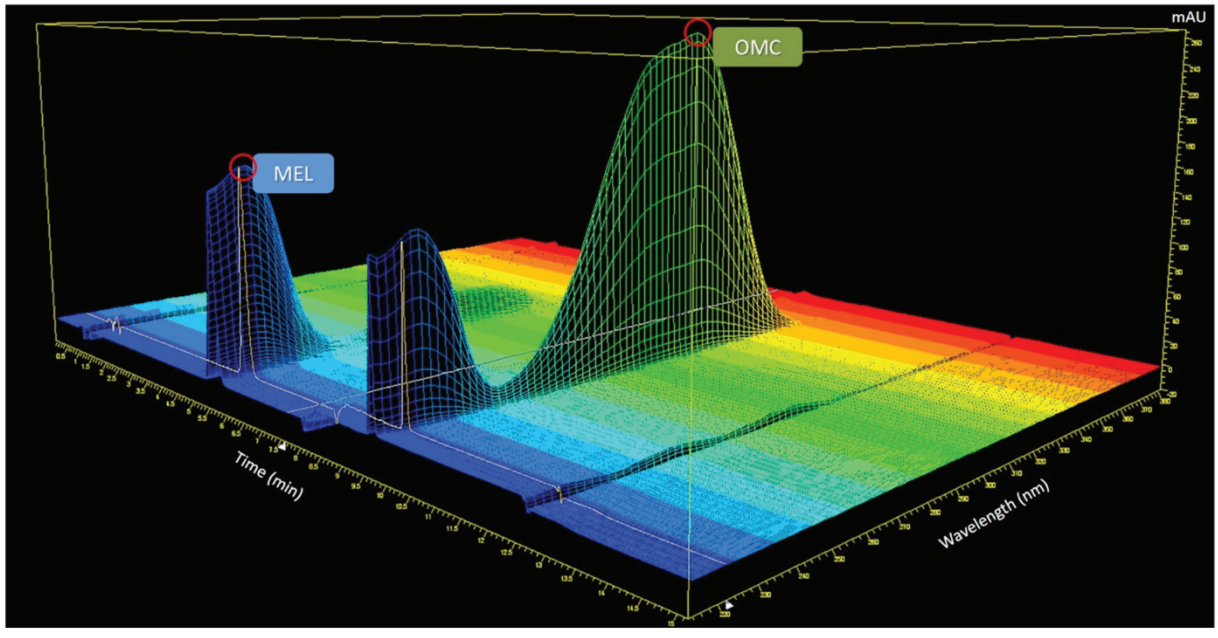

Figure 3. UV spectrum of melatonin and octyl methoxycinnamate.

The specificity of the developed method was conducted in presence of dilution solution and placebo, which is the microemulsion formulation without APIs. A representative chromatogram of MEL and OMC standard solution is shown in Figure 4(A). It is shown in Figure 4(B) and Figure 4(C) that no peak due to placebo or dilution solution was detected at the retention time of MEL and OMC. Figure 4(D) represents the sample chromatogram. MEL and OMC were extracted from the microemulsion formulation by dissolving the formulation in ethanol.

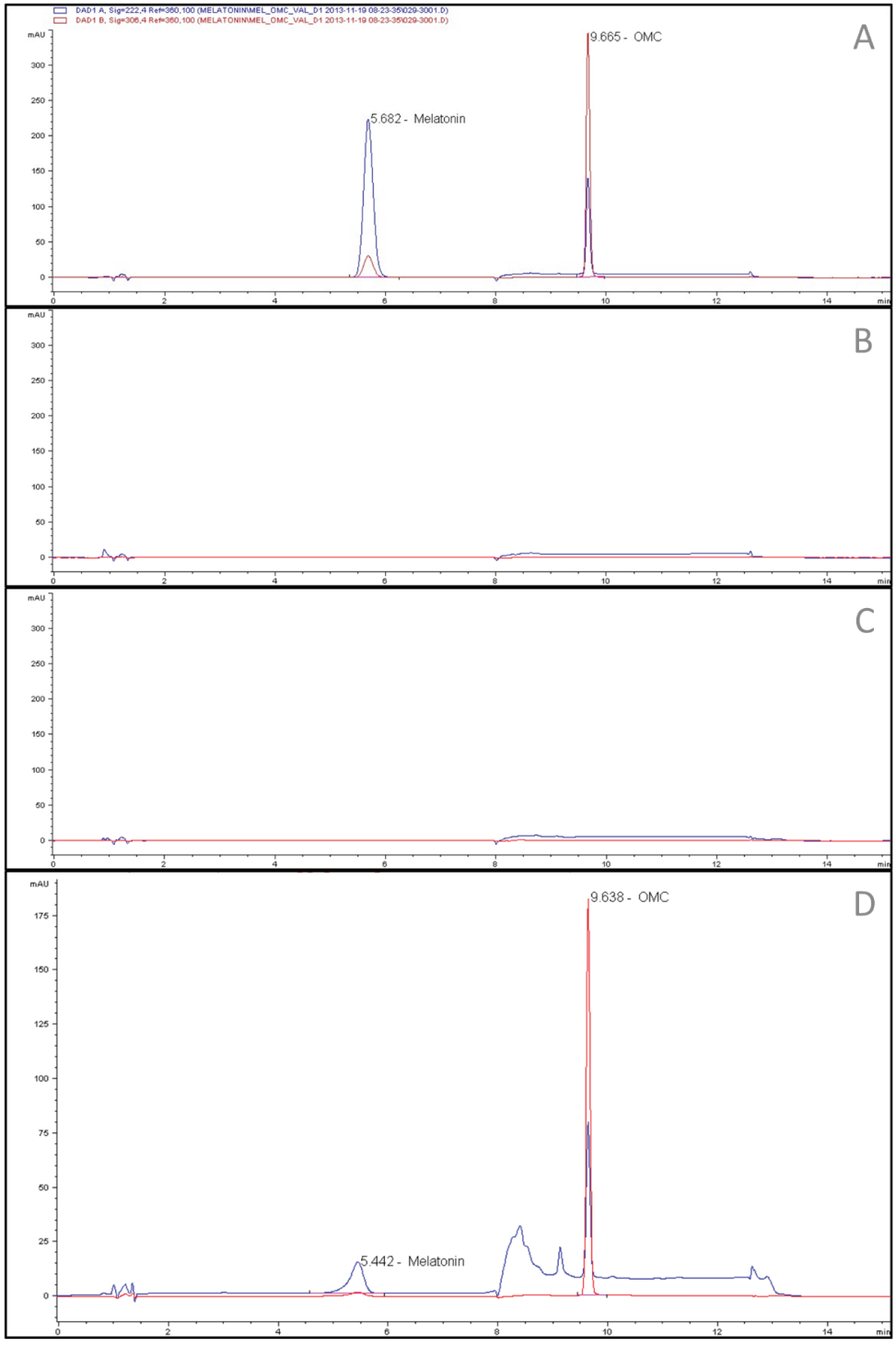

Figure 4. Chromotogram of (A) melatonin and octyl methoxycinnamate injection, (B) placebo, (C) dilution solution, and the sample chromatogram (D).

The accuracy of the method was determined by recovery experiments which were evaluated in triplicate using three concentration levels. The percentage recovery data was obtained for MEL and OMC and presented in Table 3 and Table 4, respectively. The percentage recovery data were found to be accurate for both APIs.

Table 3. Recovery of MEL.

µg/mL Added	µg/mL Found	% Recovery	Mean	SD	RSD %
40.102	40.522	101			
40.102	40.469	101	101	0.074	0.073
40.102	40.473	101			
44.092	44.735	101			
44.092	44.816	102	102	0.129	0.127
44.092	44.845	102			
42.142	42.825	102			
42.142	42.805	102	102	0.034	0.034
42.142	42.797	102			
51.462	51.961	101			
51.462	52.001	101	101	0.050	0.049
51.462	52.009	101			
51.763	52.015	100			
51.763	53.012	102	100	2.535	2.533
51.763	50.411	97			
54.187	54.272	100			
54.187	54.329	100	100	0.077	0.077
54.187	54.353	100			
63.346	63.460	100			
63.346	63.497	100	100	0.030	0.030
63.346	63.472	100			
62.851	62.945	100			
62.851	62.953	100	100	0.101	0.101
62.851	63.059	100			
67.267	67.329	100			
67.267	67.280	100	100	0.046	0.046
67.267	67.337	100			

Table 4. Recovery of OMC.

µg/mL Added	µg/mL Found	% Recovery	Mean	SD	RSD %
40.012	39.518	99			
40.012	39.518	99	99	0.020	0.020
40.012	39.532	99			
42.998	43.043	100			
42.998	43.102	100	100	0.115	0.114
42.998	43.141	100			
39.542	39.001	99			
39.542	38.974	99	99	0.034	0.035
39.542	38.989	99			
50.227	50.217	100			
50.227	50.234	100	100	0.034	0.034
50.227	50.200	100			
47.453	45.194	95			
47.453	45.383	96	96	0.378	0.396
47.453	45.553	96			
50.009	50.954	102			
50.009	51.021	102	102	0.067	0.066
50.009	50.992	102			
60.476	60.433	100			
60.476	60.469	100	100	0.050	0.050
60.476	60.493	100			
61.267	62.132	101			
61.267	62.197	102	101	0.055	0.054
61.267	62.178	101			
61.276	61.252	100			
61.276	61.264	100	100	0.040	0.040
61.276	61.299	100			

The precision of method was verified by obtaining repeatability, intermediate precision and reproducibility parameters. The results of the reproducibility of the proposed method was shown in Table 5, which was determined by preparation of six different samples from the same stock solution at the same concentration of 50 µg/ml. The results of repeatability and intermediate precision of the method was represented in Table 6. Repeatability was checked by injecting six individual sample preparations of MEL and OMC at 50 µg/ml concentration while the intermediate precision of the method was obtained by two analysts. Standard deviation (SD) and relative standard deviation (RSD) of the area for each API were calculated and evaluated.

Table 5. The results showing the reproducibility of the proposed method for precision study.

Sample	mAU	
	MEL	OMC
1	2001.6	992.8
2	2002.8	996.6
3	2004.1	1000.8
4	2005.8	1005.3
5	2007.8	1010.4
6	2008.6	1015.9
Mean	2005.12	1003.63
SD	2.78	8.65
RSD %	0.14	0.86

The linearity of the method was determined at six concentration levels (60, 40, 20, 10, 5 and 1 µg/ml). The calibration curves were plotted between the responses of peak area versus concentration of analyte. The slope and intercept value for calibration curve was y=41.787x-2.2207 (R^2=0.9995) for MEL and y=24.662x+0.5438 (R^2=0.9991) for OMC. The results reveal that an excellent correlation exists between areas and concentration of APIs within the concentration range. Calibration curves are presented in Figure 5.

Detection limit and quantification limit of the method were determined by calculating the LOD and LOQ values using the equations given above. LOD values were found 0.132 µg/ml and 0.049 µg/ml for MEL and OMC, respectively. LOQ values were determined as 0.4 µg/ml and 0.15 µg/ml for MEL and OMC, respectively.

3.3 Ex-vivo Permeation Study

Ex-vivo permeation studies showed that neither MEL nor OMC permeated to the receptor phase of the Franz diffusion cells for up to 24 hours. At the end of the permeation study, the amounts of MEL and OMC remaining on the skin were quantified. The results showed that 3.504 % MEL and 46.264 % OMC remained in the skin.

3.4 Stability Studies of the Microemulsion Formulation

The results of characterizations and 3 months stability of the proposed microemulsion formulation are presented in Table 7. Results are the mean of three replicates ± SD. The studies

Table 6. The results showing the repeatability and intermediate precision of the proposed method.

Sample	mAU			
	Analyst-1		Analyst-2	
	MEL	OMC	MEL	OMC
1	2018,60	1286,9	2099,7	1299,2
2	2190,10	1322,8	2157,2	1339,5
3	2081,70	1227,6	2105	1365,6
4	2206,20	1336,4	2235,5	1397,3
5	2236,20	1311,5	2221	1453,6
6	2197,50	1266,5	2230,7	1328,8
Mean	2155,05	1291,95	2174,85	1364
SD	77,71	36,80	62,9	55,10
RSD %	3,61	2,85	2,9	4,04

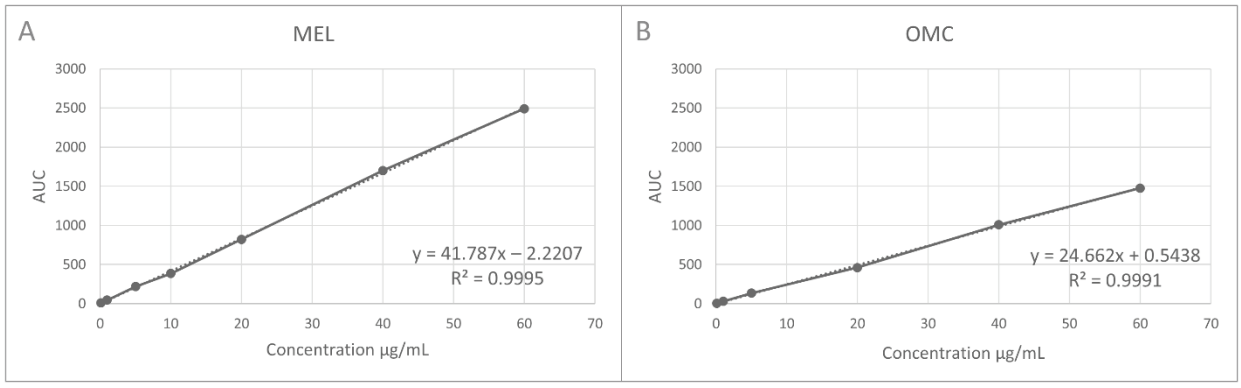

Figure 5. Calibration curves of melatonin and octyl methoxycinnamate.

Table 7. Characterizations and 3 months stability of the microemulsion formulation. Results are the mean of three replicates ± SD.

	Time	Concentration mg/ml	Conductivity mS/cm	Droplet size nm	PDI	Viscosity cP 25 °C	pH
	Tinitial	MEL: 9,754 ± 0,358 OMC: 50,188 ± 0,804	0,032 ± 0,005	193,3 ± 1,410	0,471 ± 0,032	360 ± 0.577	6,35 ± 0,025
T3 months	**4 °C**	9,842 ± 0,850 51,106 ± 0,839	0,032 ± 0,003	262,333 ± 3,955	0,408 ± 0,039	363 ± 0,862	6,45 ± 0,020
	25 °C 60 % RH	9,986 ± 2,620 51,889 ± 2,371	0,032 ± 0,003	201,466 ± 10,248	0,664 ± 0,073	960 ± 2,522	6,55 ± 0,043
	40 °C 75 % RH	9,524 ± 2,956 50,024 ± 2,438	0,033 ± 0,001	106,066 ± 2,956	0,829 ± 0,071	750 ± 3,236	6,43 ± 0,036

revealed that the proposed formulation showed good stability at 25 °C with 60 % RH in terms of API concentrations, conductivity, droplet size and pH. But the viscosity of the formulation was increased at the end of 3 months.

4. Conclusion

A simple, cost effective, specific, accurate and precise RP-HPLC method has been developed and validated for simultaneous estimation of MEL and OMC. To test the method a microemulsion formulation of MEL and OMC was also prepared and characterized. The determination coefficient for RP-HPLC method was found to be greater than 0.9990. The linearity range was found in between 0.5 and 60 µg/ml for MEL and OMC. The developed method was successfully applied to prepared microemulsion formulation and the results were found with higher confidence. The method was validated for accuracy, precision, specificity, and linearity according to guidelines. Also the method was successfully used for determination API concentration in *ex-vivo* permeation and stability studies of the proposed microemulsion formulation composed of MEL and OMC. In conclusion, this method can be used for determination of MEL and OMC in pharmaceutical formulations.

Conflicts of interest

The authors reveal no conflict of interest in this manuscript.

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References

1. Slominski A, Fischer TW, Zmijewski MA, Wortsman J, Semak I, Zbytek B, Slominski RM, Tobin DJ. On the role of melatonin in skin physiology and pathology. Endocr 2005; 27: 137-48.
2. Slominski AT, Kleszczyński K, Semak I, Semak I, Janjetovic Z, Zmijewski MA, Kim TK, Slominski RM, Reiter RJ, Fischer TW. local melatoninergic system as the protector of skin integrity. Int J Mol Sci 2014; 15: 17705-32.

3. Sierra AF, Ramírez MLG, Campmany ACC, Martínez AR, Naveros BC. *In vivo* and *in vitro* evaluation of the use of a newly developed melatonin loaded emulsion combined with UV filters as a protective agent against skin irradiation. J Dermatol Sci 2013; 69: 202-14.
4. Marto J, Ascenso A, Gonçalves LM, Gouveia LF, Manteigas P, Pinto P, Oliveira E, Almeida AJ, Ribeiro HM. Melatonin-based pickering emulsion for skin's photoprotection. Drug Deliv 2016; 23: 1594-607.
5. Wang SQ, Lim HW. Current status of the sunscreen regulation in the United States: 2011 Food and Drug Administration's final rule on labeling and effectiveness testing. J Am Acad Dermatol 2011; 65: 863-9.
6. Heuschkel S, Goebel A, Neubert R.H.H. Microemulsions—modern colloidal carrier for dermal and transdermal drug delivery. J Pharm Sci 2008; 97: 603–31.
7. Lopes LB. Overcoming the cutaneous barrier with microemulsions. Pharmaceutics 2014; 6: 52-77.
8. Carlotti ME, Gallarate M, Rossatto V. O/W microemulsion as a vehicle for sunscreens. J Cosmet Sci 2003; 54: 451–62.
9. Lin J, Zhang C, Gao Y, Zhao X, Li X. A validated HPLC method for determining melatonin in capsule dosage form. Spatula DD 2012; 2: 147-51.
10. Kedor-Hackmann ERM., De Lourdes Pérez González ML, Singh AK, Santoro MIRM. Validation of a HPLC method for simultaneous determination of five sunscreens in lotion preparation. Int J Cosmet Sci 2006; 28: 219–24.
11. Andrisano V, Bertucci C, Battaglia A, Cavrini V. Photostability of drugs: Photodegradation of melatonin and its determination in commercial formulations. J Pharm Biomed Anal 2000; 23: 15-23.
12. ICH (Q2 (R1); Validation of Analytical Procedures: Text and methodology. International Conference on Harmonization, Geneva. 2005.
13. Songkro S, Lo NL, Tanmanee N, Maneenuan D, Boonme P. *In vitro* release, skin permeation and retention of benzophenone-3 from microemulsions (o/w and w/o). J Drug Deliv Sci Technol 2014; 24: 703-11.
14. FDA, Reviewer Guidance—Validation of chromatographic methods. Rockville, United States of America. 1994.