Faculty of Pharmacy, Department of Pharmaceutical Technology, Anadolu University, Eskisehir, Turkey

A validated HPLC method for the determination of octocrylene in solid lipid nanoparticle systems

M. S. BERKMAN, Y. YAZAN

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Assist. Prof. Murat Sami Berkman, Faculty of Pharmacy, Department of Pharmaceutical Technology, Anadolu University, 26470, Eskişehir, Türkiye
muratsb@anadolu.edu.tr

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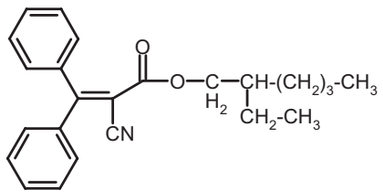
UV filters are traditionally classified as chemical absorbers and physical blockers depending on their mechanism of action. In this study, one of the most important chemical UVB absorber, octocrylene, was incorporated into Solid Lipid Nanoparticle (SLN) systems which themselves have UV blocking potential similar to physical blockers. Determination of octocrylene in the formulations was performed by HPLC (High Performance Liquid Chromatography) using a new validated method based on ICH harmonised tripartite guideline "validation of analytical procedures Q2(R1)". Determination and validation studies were carried out on a 4.6 × 250 mm, 5 μm C_{18} ACE column using an optimized mobile phase of acetonitrile:water (75:25, v/v) at a flow rate of 1.5 mL·min^{-1}. UV detection was performed at 210 nm and the column temperature was adjusted to 50 °C. Cyclosporine A was used as an internal standard (IS). The specified working range was derived from linearity studies and kept in the concentration range of 2.5 ×– 5.5 × 10^{-5} M. Good correlation and accuracy were obtained. Limit of detection (LOD) and limit of quantitation (LOQ) values were determined to be 1.64 × 10^{-6} M and 4.97 × 10^{-6} M, respectively. Octocrylene recovery % results of the SLN formulations stored at 25 °C, 4 °C and 40 °C for 360 days were investigated and compared to the freshly prepared samples.

1. Introduction

A sunscreen product is any preparation intended to be placed in contact with human skin with the exclusive or main objective being the protection of the skin from UV radiation through absorbing, scattering or reflecting. Sunscreens provide this UV protection either by chemical absorbers (absorbing) or physical blockers (scattering and reflecting) like titanium oxide and zinc oxide, or a combination of both (Poiger et al. 2004; Simeoni et al. 2005).

Due to their particulate character, SLNs act as physical sunscreens on their own. Incorporation of molecular sunscreens into these carrier systems is possible and leads to long-term stable formulations and synergistic UV-blocking effects (Wissing and Müller 2003; Demirel and Yazan 2000). Therefore, the concentration of molecular sunscreens in a formulation can be decreased while maintaining the protection level (Wissing and Müller 2001).

In this study, one of the most important chemical absorber, octocrylene, was incorporated into a SLN system (Anderson et al. 1997). Octocrylene (2-ethylhexyl-2-cyano-3,3-diphenylacrylate) is a UVB absorber and the approved use level in the U.S. is up to 10% alone and 7–10% when used in combination with other sunscreening agents. It is an excellent ingredient for water resistant sunscreen products and with high SPF formulations (Salvador and Chisvert 2005).



Octocrylene

2. Investigations, results and discussion

Method validation was conducted and accuracy and reliability was proven. The specificity of the method was analyzed for SLN systems; it was determined that overlap effects of other components in the formulation did not affect IS peaks and active ingredients.

Intra-day and inter-day linear regression analysis was performed on the series prepared for linearity determination and the results are portrayed in Table 1.

The mean PN ratios of the three series were plotted versus OCR concentration values to calculate the linearity equation as shown in Fig. 1.

For the accuracy test, samples containing three different concentrations within the operational range of OCR (approximately 3 × 10^{-5}, 4 × 10^{-5} ve 5 × 10^{-5} M) were prepared and this method yielded high recovery (Table 2).

Table 1: Intra-day and inter-day (pool-days) linearity results

	Intra-day (n = 5)			Inter-day (n = 15)
	Day 1	Day 2	Day 3	Mean
Slope	14298.21	14509.52	14792.67	14533.47
Slope SD	142.3018	101.9899	96.3481	195.3594
y-intercept	−0.0027	0.0037	0.0085	0.0032
y-intercept SD	0.0071	0.0051	0.0048	0.0098
CI 95%	±452.81	±324.54	±306.58	±421.98
R	0.9999	0.9999	0.9999	0.9988
r^2	0.9997	0.9999	0.9999	0.9977

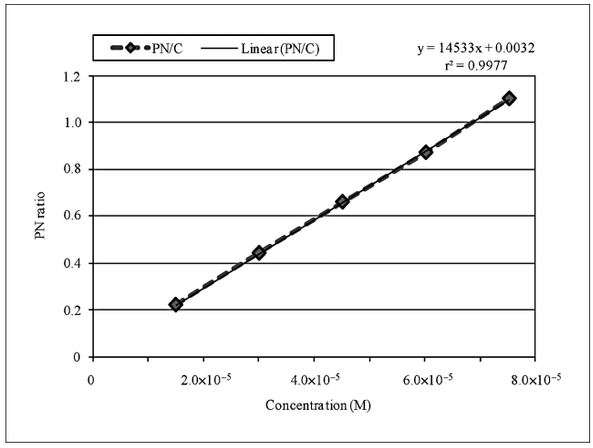


Fig. 1: OCR linearity in acetonitrile:water (75:25)

To portray the intra-day repeatability of the method, 3 different concentrations of OCR containing samples were prepared and 6 replicates were performed for each concentration. For inter-day repeatability, the same series' were prepared on different days. Results were provided as standard error and relative standard deviation (coefficient of variation). Intra- and inter-day repeatability results are given in Tables 3–5

Measurements performed on three different concentrations (low, medium, and high) evaluating the repeatability and reproducibility of the analytical method used seem to verify the precision of the method since the coefficient of variation (VC) % is below 2%. The method precision was found to be within the targeted intervals according to the repeatability and reproducibility tests.

The specificity of the method was determined by placebo analyses. For this study, placebo SLN formulations were prepared. Sample preparations was processed through the steps taken for quantity determination. Any interferrence on OCR specificity with other components was investigated using the chromatograms obtained.

The chromatograms obtained showed that OCR and IS peaks are distinctly separated (Fig. 2). It was therefore concluded that the method used is specific.

Table 2: Series prepared for determination of accuracy and recovery results (n = 6)

OCR (M)	Mean PN_{RATIO}	Recovery (M)	Recovery %	SE	RSD
3.01×10^{-5}	0.4415	3.02×10^{-5}	100.21	0.30	0.74
4.06×10^{-5}	0.5915	4.05×10^{-5}	99.64	0.34	0.84
4.97×10^{-5}	0.7247	4.96×10^{-5}	99.97	0.23	0.55

Table 3: Intra-day linearity precision and accuracy results (n = 6)

OCR (M)	Mean PN_{RATIO}	Recovery (M)	Recovery %	RSD	SE
1.50×10^{-5}	0.2229	1.51×10^{-5}	100.47	0.60	0.25
4.51×10^{-5}	0.6562	4.49×10^{-5}	99.54	0.55	0.23
7.52×10^{-5}	1.0991	7.54×10^{-5}	100.22	0.65	0.26

Table 4: Inter-day linearity precision and accuracy results (n = 6)

OCR (M)	Mean PN_{RATIO}	Recovery (M)	Recovery %	RSD	SE
1.50×10^{-5}	0.2212	1.50×10^{-5}	99.68	0.84	0.34
4.51×10^{-5}	0.6574	4.50×10^{-5}	99.71	0.81	0.33
7.52×10^{-5}	1.0972	7.53×10^{-5}	100.05	0.49	0.20

Table 5: Intra-day and inter-day (pool-days) precision results on single concentration

	Intra-day (n = 6)			Inter-day Mean (n = 18)
4×10^{-5} M	Day 1	Day 2	Day 3	
Mean PN_{RATIO}	0.6420	0.6668	0.6738	0.6608
RSD	0.5507	1.1331	1.0660	2.3108
SE	0.0014	0.0031	0.0029	0.0036
CI 95%	±0.0037	±0.0079	±0.0075	±0.0076

Chromatograms of the calibration set prepared within the OCR were used for the calculation of LOD and LOQ values. The obtained values were:

$$\text{LOD} = 3.3\sigma/\text{S} = (3.3 \times 0.007102274)/14298.21$$
$$= 1.64 \times 10^{-6}\text{M}$$

$$\text{LOQ} = 10\sigma/\text{S} = (10 \times 0.007102274)/14298.21$$
$$= 4.97 \times 10^{-6}\text{M}$$

(σ = standard deviation of responce;
s = slope of the calibration curve)

1.64×10^{-6} M and 4.97×10^{-6} M concentrations calculated are high for LOD and LOQ values.

This may be attributed to the following:

- use of PDA detector
- high content of organic solvent and partial UV-*cutoff* effect
- using λ_{maks} to capture IS and active ingredient at the same time

The lowest concentration level used in this method is 2.5×10^{-5} M. Since LOD and LOQ values are less than this concentration, it can be colcluded that the method used is sensitive.

A series of system suitability parameters such as mobile phase, flow rate, injection volume, oven temperature, etc. was established to ensure the validity of the analytical procedure whenever it is used.

Methanol:acetonitrile:water, methanol:acetonitrile and acetonitrile:water mixtures at different ratios were tested as the mobile phase. Acetonitrile:water (75:25) was selected as the mobile

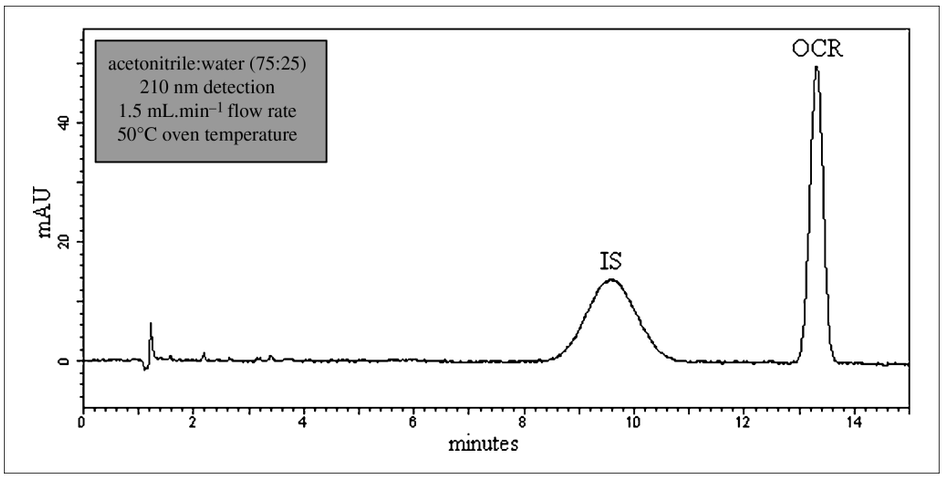


Fig. 2: IS and OCR chromatogram

phase due to appropriate retention time, good separation and distances of active ingredient and IS peaks.

Flow rate of the mobile phase was investigated in the range of 1–2 mL.min^{-1} according to the characteristics of the column. A flow rate of 1.5 mL·min^{-1} was selected among the others depending on the most appropriate peak shape and retention time.

Injection volume of 20 μL was selected within the range of 10–20 μL and used in all validation studies. Solvent front was observed approximately at 1.2 minutes.

Column volume was calculated by multiplying the flow rate with solvent front time.

$$Column\ Volume = 1.5\,mL.\text{min}^{-1} \times 1.2\,\text{min} = 1.8\,mL$$

Approximately 1% of the column volume (18 μL) was used as the injection volume.

When oven temperature was tested in the range of 25–60 °C, it was observed that IS and OCR displayed sharper peak symmetry by the increase in temperature. It was also found that the base of the peak was enlarged when IS was injected at room temperature and degradation products of OCR started to form when the oven temperature was increased to 60 °C. However, increased temperature showed no effect on IS retention time while retention time of OCR was decreased. An oven temperature of 50 °C was found to be optimal and the studies were carried out at this temperature.

Due to the apolar structure of the substance, 4.6 × 250 mm, 5 μm C_{18} ACE column was preferred. To reduce the retention times and to ensure the best separation of the peaks from each other, 4.6 × 150 mm, 5 μm Zorbax Eclipse XDB- C_8 column which has a shorter length and shows less apolar properties then the aforementioned ACE column was additionally tested. As a result of these tests, the ACE column was preferred in the studies.

Cyclosporine A, phenobarbital and paracetamol were tested as IS materials. Peak affinity of the active ingredient and IS, retention times and the peaks in a common wavelength were examined for each material. As a result of preliminary testing, cyclosporin A was determined to be used in the studies.

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system to be evaluated.

Equipment, brand, model, calibration and operation information for system suitability testing are shown in Table 6.

OCR and IS peak morphologies were calculated by the software of the HPLC device using the chromatograms derived from the investigations (Fig. 3 and Table 7). A Shimadzu HPLC device in AÜBİBAM (Medicinal Plants, Drugs and Scientific Research Center) was used for the HPLC process validation and active ingredient determinations.

Codes of the SLN formulations used during quantification studies are presented in Table 8.

The final SLN formulation consisted of 6% solid lipid and the active ingredient [10% of the solid lipid mass]. Theoretically, 0.18 g OCR was loaded into the 30 g of formulations.

Free, superficial and loaded amounts of the active ingredient were found to be 2.47%, 87.15% and 9.36%, respectively for the freshly prepared formulations (Table 9).

Table 6: Equipments, brands, models of the HPLC

Equipments	Brands	Models
Degasser	Shimadzu	DGU-14 A
Pump	Shimadzu	LC-10 AT VP
System control	Shimadzu	SCL-10 A VP
Auto-injector	Shimadzu	SIL-10 AD
Diode array dedector	Shimadzu	SPD-MID A VP
Column oven	Shimadzu	CTO-10 AC VP

Table 7: Peak morphology results of OCR formulations

Parameters	IS	OCR
Asymmetry factor	1.05	0.96
Capacity factor	6.80	9.87
Peak area RSD%	0.83	0.92
Theoretical plate number	12886.54	
Selectivity factor	1.440804	
Resolution factor	16.03	

Table 8: Codes of the SLN formulations prepared

FSO-T0	Freshly prepared OCR loaded SLN
FSO-T360 4 °C	OCR loaded SLN after 360-day storage at 4 °C
FSO-T360 25 °C	OCR loaded SLN after 360-day storage at 25 °C
FSO-T360 40 °C	OCR loaded SLN after 360-day storage at 40 °C

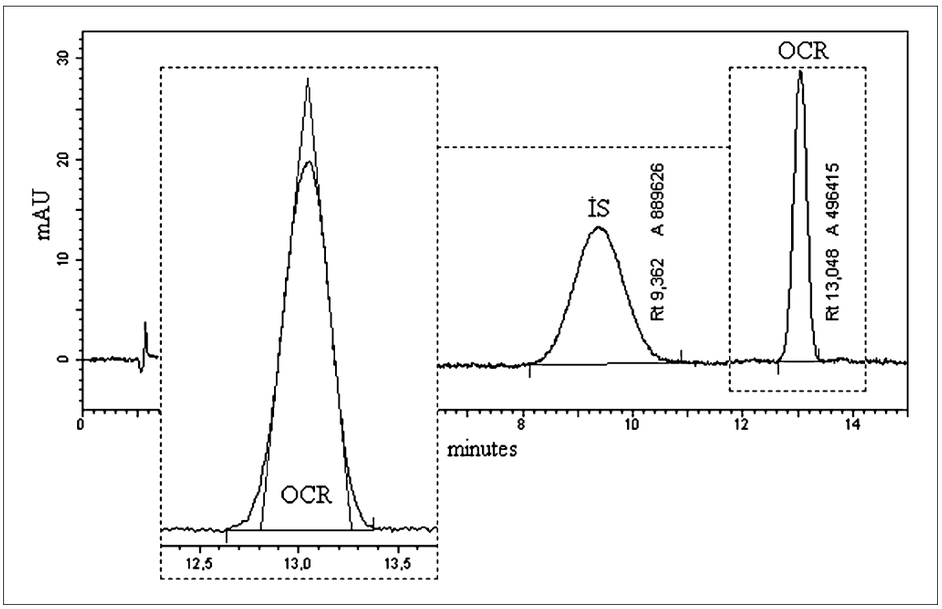


Fig. 3: Calculation of peak morphologies using chromatograms

Total recovery % was found to be 98.98% for the freshly prepared SLNs. The reason for 1.02% loss in active ingredient may be due to any sticking to the beaker or stirrer during the preparation stage.

After a 360-day storage period, the free OCR quantity increased from 2.47% to 3.22% (25 °C), 3.23% (4 °C) and 7.37% (40 °C). This may be due to the slow transfer of the superfacial active ingredient to the aqueous phase.

The superfacial OCR quantity of 87.15% decreased to 79.54% (25 °C), 78.73% (4 °C) and 72.15% (40 °C) after a 360-day storage period. The reason of this decrease may be the aforementioned slow transfer of the active ingredients to the aqueous phase or any stability problem.

OCR quantity loaded was found to be 9.36% for freshly prepared SLNs. The quantity after 360 days changed to 9.06% (25 °C), 8.74% (4 °C) and 6.19% (40 °C) which may be due to the transfer of the active ingredient through the phases or possible stability problems.

Comparison of OCR recovery % results from the SLN formulations stored at 25 °C, 4 °C and 40 °C for 360 days and the freshly prepared samples are demonstrated in Fig. 4.

Table 9: Recovery % of OCR from SLNs

	Codes	Recovery % ± SE	RSD	CI 95%
Free	FSO-T0	2.47 ± 0.01	1.15	± 0.03
	FSO-T360 4 °C	3.23 ± 0.01	0.60	±0.02
	FSO-T360 25 °C	3.22 ± 0.02	1.19	± 0.04
	FSO-T360 40 °C	7.37 ± 0.02	0.77	± 0.06
Superficial	FSO-T0	87.15 ± 0.17	0.49	± 0.45
	FSO-T360 4 °C	78.73 ± 0.20	0.64	± 0.52
	FSO-T360 25 °C	79.54 ± 0.21	0.66	± 0.55
	FSO-T360 40 °C	72.15 ± 0.25	0.86	± 0.66
Loaded	FSO-T0	9.36 ± 0.24	6.23	± 0.61
	FSO-T360 4 °C	8.74 ± 0.35	9.95	± 0.91
	FSO-T360 25 °C	9.06 ± 0.32	8.74	± 0.83
	FSO-T360 40 °C	6.19 ± 0.35	13.77	± 0.89

n = 6 SE: standard error RSD: relative standard deviation CI: confidence interval

3. Experimental

3.1. Materials

Solid lipid tripalmitin (Dynasan® 116; m.p. 63 °C) was purchased from Fluka Chemie GmbH (Switzerland) and polyoxyethylene sorbitan monooleat (Tween® 80) from Merck Schuchardt (Germany). UV filter octocrylene (Eusolex® OCR) was provided by Merck KgaA (Germany). The mobile phase, analytical-reagent grade acetonitrile for the high performance liquid chromatography were purchased from Merck KgaA (Germany) and used with no purification. The internal standard cyclosporine A was supplied by Novartis (Türkiye).

3.2. Preparation of nanoparticles

The melting point measurements of Dynasan® 116 were conducted using a basic melting point device (Electrothermal 9100, USA) with capillary tubes and a differential scanning calorimeter (Shimadzu DSC 60, Japon). Measurements were repeated 3 times and the average melting point was found to be 63 °C. For empty formulations, the hot aqueous surfactant solution was dispersed in the molten solid lipid at elevated temperatures and stirred by a high-performance dispersing instrument to get a hot oil-in-water emulsion. Speed range was varied between 3–5 min. at 8000–13500 rpm using an ultra-turrax (Janke&Kunkel® IKA, Labortechnik, Germany). In order to prepare nanoparticles, the lipid phase was melted at 10 ± 1 °C above the melting point of the solid lipid and the temperature was set at 73 ± 1 °C in a thermostated water bath during stirring. 20–40 g of nanoemulsion pre-mixtures prepared in 50 mL small glass beakers were then isolated from external effects and cooled down to room temperature. SLNs were formed after recrystallization. Following the optimization of empty formulations, OCR (octocrylene)−10% of the solid lipid mass- was dispersed in the molten solid lipid phase and the aforementioned procedure was applied. This pre-mixture was then homogenized using a high pressure homogenizer APV-2000

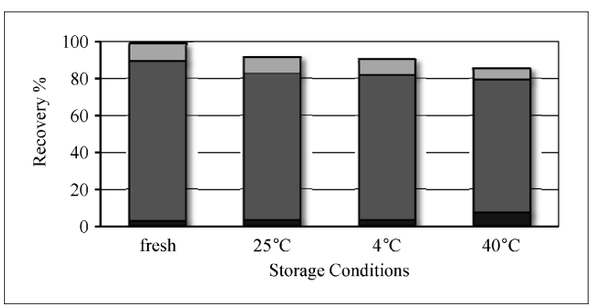


Fig. 4: Recovery % OCR from the SLNs loaded superficial free

Table 10: Dynasan® 116-based SLN formulations

Code	Empty Formulation FSE	OCR Formulation FSO
Dynasan® 116	6%	6%
Tween® 80	4%	4%
Active Ingredient	–	0.6%
Distilled Water	90%	89.4%
Stirrer	ultraturrax	ultraturrax
Temperature	73 °C	73 °C
Stirring rpm	13 500	13 500
Stirring Time	5 min	5 min
Homogenization	500 bar 3 cycles	500 bar 3 cycles
Particle size	100–150 nm	100–150 nm

(Denmark) applying three homogenization cycles at 500 bar. Table 10 provides an overview of the SLN formulations prepared.
OCR recovery % results of the SLN formulations stored at 25 °C, 4 °C and 40 °C for 360 days were investigated and compared with the freshly prepared samples. HPLC was used to determine the OCR quantity in the SLN formulations prepared.

3.3. HPLC method validation

The Analytical Process Validation Method Q2(R1) of the International Harmonization Committee was used in this study and the parameters such as linearity, accuracy, precision and specificity were evaluated (ICH Q2B 1996, ICH Q2(R1) 2005).
The operating conditions applied during the validation process are given in Table 11.
Cyclosporine A was selected as internal standard (IS). 10.1 mg accurately weighed cyclosporine A was mixed with the mobile phase to obtain a 50 mL [0.202 mg.mL^{-1} (**A**)] solution. 0.2 mL of this stock solution was used for each measurement with varying concentrations of the active ingredient and the mobile phase to obtain 1 mL of solution. The molarity calculation of the IS solution prepared is as follows:

$$0.202 \times 0.2 = 0.0404\,mg\,(0.2\,mL)$$

$$0.0404\,mg \cdot mL^{-1} = 0.0404\,g \cdot L^{-1}$$

$$M = n/V = 0.0404/1202.6 = 3.4 \times 10^{-5}\ M\ IS$$

where M = molarity; n = mol; V = volume (L)
50 mL of active ingredient solution was prepared by mixing 27.2 mg of accurately weighed OCR with the mobile phase to obtain a 1:10 dilution [0.054 mg·mL^{-1} (**B**)]. Appropriate concentrations in the range of 0.1–0.5 mL taken from the stock solution, mobile phase and 0.2 mL IS solution were used to obtain 1 mL samples.
The molarity calculations of the active ingredient solution prepared from 0.1 mL **B** stock solution is given below:

$$0.054 \times 0.1 = 0.0054\,mg\,(0.1\,mL)$$

$$0.0054\,mg \cdot mL^{-1} = 0.0054\,g \cdot L^{-1}$$

$$M = n/V = 0.0054/361.5 = 1.5 \times 10^{-5} M\ OCR$$

where M = molarity; n = mol; V = volume (L)
For the establishment of linearity, a minimum of 5 concentrations is recommended. The stock solutions **A** and **B** comprising of IS and OCR in mobile phase were used to prepare 5 sample solutions of varying OCR concentration. IS concentration was kept constant at 3.4×10^{-5} M for all solutions.
Three separate series of the same concentration were prepared for the determination of linearity. Preparation of the OCR series in the range of 1.5×10^{-5}–7.5×10^{-5} M is shown below:

$$0.2\,mL\ \boldsymbol{A} + 0.1\,mL\ \boldsymbol{B} + 0.7\,mL\ mobile\ phase\ (1.5 \times 10^{-5} M\ OCR)$$

$$0.2\,mL\ \boldsymbol{A} + 0.2\,mL\ \boldsymbol{B} + 0.6\,mL\ mobile\ phase\ (3.0 \times 10^{-5} M\ OCR)$$

$$0.2\,mL\ A + 0.3\,mL\ B + 0.5\,mL\ mobile\ phase\ (4.5 \times 10^{-5} M\ OCR)$$

$$0.2\,mL\ \boldsymbol{A} + 0.4\,mL\ \boldsymbol{B} + 0.4\,mL\ mobile\ phase\ (6.0 \times 10^{-5} M\ OCR)$$

$$0.2\,mL\ \boldsymbol{A} + 0.5\,mL\ \boldsymbol{B} + 0.3\,mL\ mobile\ phase\ (7.5 \times 10^{-5} M\ OCR)$$

The series prepared were injected into the HPLC and the individual peak normalizations (PN) for IS and OCR were calculated using the area of chromatograms obtained and the retention times (Rt).

$$PN_{IS} = Area_{IS}/Rt_{IS}$$
$$PN_{OCR} = Area_{OCR}/Rt_{OCR}$$
$$PN_{RATIO} = PN_{OCR}/PN_{IS}$$

The mean PN ratios of the three series versus the OCR concentration values were used to calculate the linearity equation.
Chromatograms of the calibration set prepared within the OCR concentration range of 1.5×10^{-5}–7.5×10^{-5} M can be seen in Fig. 5

Table 11: Operating conditions of HPLC

Mobile phase	Acetonitrile:Water (75:25, v/v)
Injection volume	20 µL
Oven temperature	50 °C
Flow rate	1.5 mL.min^{-1}
Column	4.6 × 250 mm, 5 µm C_{18} ACE
Detection	210 nm

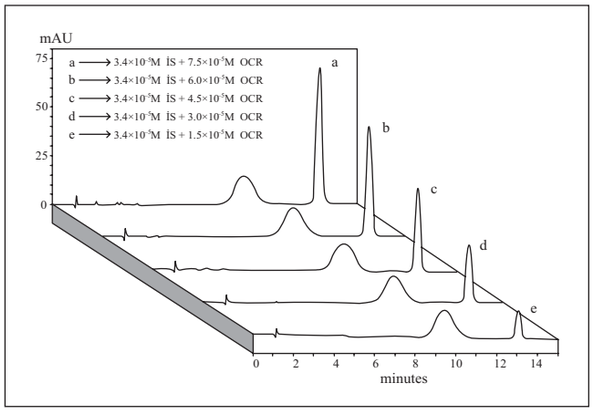


Fig. 5: Chromatograms of the calibration set prepared in the concentration range of 1.5×10^{-5}–7.5×10^{-5} M Eusolex® OCR

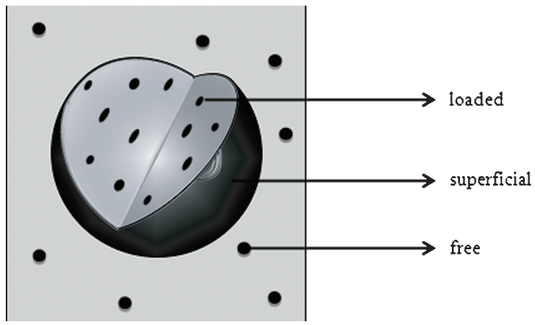


Fig. 6: Schematic representation of the active ingredient on a single nanoparticle

3.4. Sensitivity

In the present work, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated according to the referred ICH guideline.
A calibration curve was prepared using samples containing OCR and IS. The standard deviation of y-intercepts of regression lines was used as σ (standard deviation of the response).
Specified working range was derived from the linearity studies and kept in the concentration range of 2.5×10^{-5}–5.5×10^{-5} M (60–135% of the active ingredient in the formulations).

3.5. Preparation of samples

Three different extraction methods were used for the determination of the free, superficial and loaded OCR (Fig.6). Experiments were repeated 6 times for each sample and the amount of OCR in the formulations were expressed as % recovered. Standard error (SE), relative standard deviation (RSD) and 95% confidence interval (CI) values were also calculated.

3.6. Determination of free OCR

Accurately weighed SLN suspension 1 mL was diluted with 9 mL distilled water. 1 mL of this suspension was then added to 0.6 mL mobile phase with 0.4 mL IS to obtain a 2 mL solution and filtered through a 0.1 μm polypropylene filter. 1 mL of this transparent filtrate was applied to the column in order to calculate the amount of free (in water) OCR in the suspension.

3.7. Determination of superficial OCR

Approximately 30 mg of SLN suspension was mixed with ethanol to obtain a 10 mL suspension. The suspension was then centrifuged at 1000 rpm for 10 min following 5 min in an ultrasonic bath at 25 °C. 1 mL of the transparent portion of the suspension was added to 0.6 mL mobile phase with 0.4 mL IS to obtain a 2 mL solution and filtered through a 0.1 μm polypropylene. 1 mL of this transparent filtrate was applied to the column to calculate the amount of the superficial OCR simultaneously with the free portion in water. The amount of free OCR was then subtracted from the calculated amount to obtain the superficial OCR.

3.8. Determination of OCR loaded

Approximately 30 mg of SLN suspension was mixed with ethanol to obtain a 10 mL suspension. The suspension was then centrifuged at 1000 rpm for 10 min following 5 min in an ultrasonic bath at 70 °C. If there was any reduction in the amount due to the temperature applied, it was once again mixed with ethanol to obtain a 10 mL suspension. 1 mL of the transparent portion of the suspension was added to 0.6 mL mobile phase with 0.4 mL IS to obtain 2 mL of solution and filtered through a 0.1 μm polypropylene filter. 1 mL of this transparent filtrate was applied to the column to calculate the total OCR in the formulation. The amount of free and superficial OCR were then substracted from the total OCR amount to obtain the OCR loaded.

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