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Self-assembled polymeric nanocarriers for the targeted delivery of retinoic acid to the hair follicle

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SUPPLEMENTARY INFORMATION

1 Validation of HPLC-UV method for quantification of RA in micelles

1.1 Specificity

The method was considered to be specific for retinoic acid (all RA isomers) as it eluted at 2.25 min and its peak was clearly separated from solvent and copolymer signals 0-1.5 min). Figure SI1 presents the chromatograms obtained for RA standard, unloaded micelles and RA loaded micelle sample.

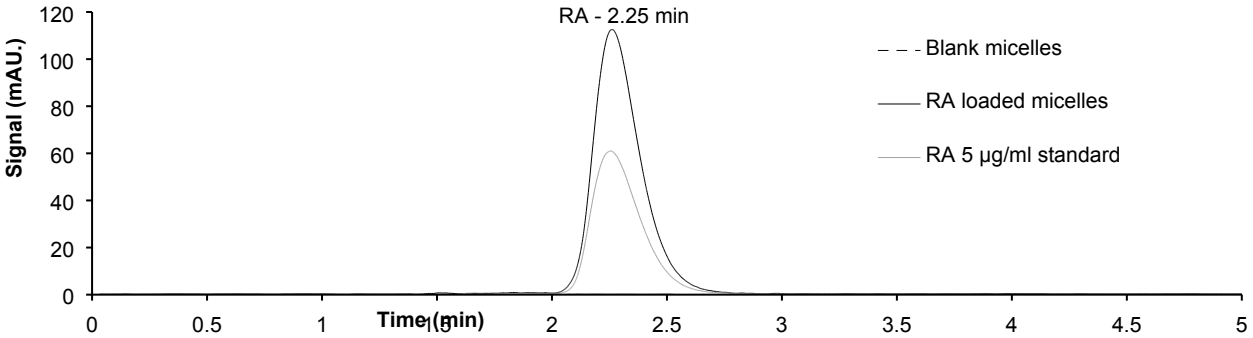

Figure SI1: Chromatograms of RA standard (5 µg/ml), unloaded micelles and RA loaded micelle samples.

1.2 Limit of detection and limit of quantification

The lowest limit of detection (LOD) and lowest limit of quantification (LOQ) were determined using the standard deviation of the response and slope method. They were found to be 0.75 µg/ml and 2.26 µg/ml, respectively.

1.3 Precision and accuracy

Intra- and inter-day precision and accuracy was assessed using 5, 10 and 50 µg/ml standards. Table SI1 shows a summary of intra- and inter-day accuracy and precision for the method.

Table SI1: Intra- and inter-day precision and accuracy for quantification of RA in micelles.

	Intra-day			Inter-day 1			Inter-day 2		
$[RA]_{theo}$ (ug/ml)	$[RA]_{meas}$ (ug/ml)	RSD (%)	Recovery (%)	$[RA]_{meas}$ (ug/ml)	RSD (%)	Recovery (%)	$[RA]_{meas}$ (ug/ml)	RSD (%)	Recovery (%)
5	4.75 ± 0.02	0.38	94.92	4.56 ± 0.23	5.08	91.17	4.59 ± 0.23	4.94	91.76
10	9.69 ± 0.04	0.45	96.94	9.35 ± 0.41	4.35	93.54	9.33 ± 0.46	4.96	93.34
50	49.38 ± 0.29	0.59	98.75	47.71 ± 1.29	2.70	95.42	47.91 ± 1.62	3.38	95.82

The results indicate that for intra-day measurements, the mean recoveries ranged from 94.92 to 98.75 % (RSD 0.38 –0.59 %). The mean recoveries for inter-day analysis on day 1 were between 91.17 and 95.42 % (RSD 2.70 – 5.08 %) and on day 2 they ranged from 91.76 to 95.82 % (RSD 3.38 – 4.96 %). The method was considered as accurate and precise as all measured values were within the acceptance limits of ICH (2005)[1] and FDA Bioanalytical Method Validation (2001) Guidelines[2].

2 Validation of UHPLC-MS/MS method for quantification of RA in skin samples

2.1 Specificity

Figure SI2 shows the MRM traces of a) RA and ACT standards and b) both traces in a blank skin extract and c) both traces in skin samples.

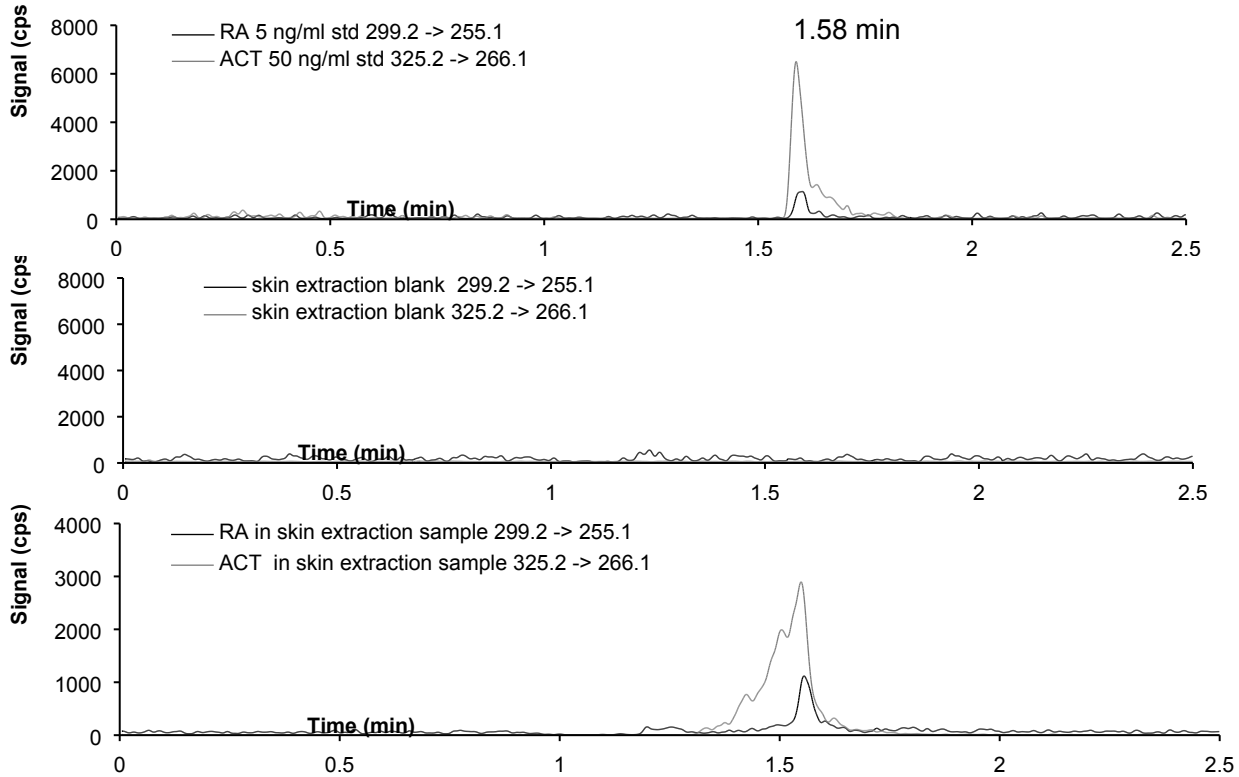

Figure SI2: Respective MRM traces of a) RA and ACT standards b) both traces in a blank skin extract and c) both traces in skin samples

No RA signal was found in blank skin permeation and extraction samples. The method was considered to be specific for RA quantification in skin samples

2.2 Linearity

Calibration curves were constructed by plotting the ratio of RA and ACT peak area (cps/min) against the ratio of RA and ACT nominal concentrations (ng/ml). A good linear fit was found in the concentration range of 1 – 200 ng /ml. Correlation coefficients for all calibration curves were superior to 0.98.

2.3 Limit of detection and limit of quantification

The lowest concentrations of RA to be detected (LOD) and lowest limit of RA quantified (LOQ) were found to be 1.65 ng/ml and 5.00 ng/ml, respectively.

2.4 Precision and accuracy

Intra- and inter-day precision and accuracy was assessed using 5, 100 and 1000 ng/ml RA solutions in acetonitrile skin extracts. Table SI2 shows a summary of intra- and inter-day accuracy and precision for the method.

Table SI2 Intra- and inter-day precision and accuracy for quantification of RA in skin samples

	Intra-day			Inter-day 1			Inter-day 2		
$[RA]_{theo}$ (ng/ml)	**$[RA]_{meas}$ (ng/ml)**	**RSD (%)**	**Recovery (%)**	**$[RA]_{meas}$ (ng/ml)**	**RSD (%)**	**Recovery (%)**	**$[RA]_{meas}$ (ng/ml)**	**RSD (%)**	**Recovery (%)**
5	4.9 ± 0.5	0.1	95.1	5.0 ± 0.3	5.9	99.1	4.6 ± 0.5	10.8%	92.9%
100	92.0 ± 7.4	0.1	92.0	93.1 ± 7.4	8.0	93.1	93.2 ± 11.4	12.2%	93.2%
1000	954.0 ± 35.5	0.1	95.4	963.8 ± 58.6	6.1	96.1	1017 ± 131.3	12.9%	101.7%

The mean recoveries for intra-day measurements observed ranged from 92.0 to 95.4% (RSD 0.12 - 0.14 %). The mean recoveries for inter-day analysis on day 1 were between 93.1 and 99.1% (RSD 5.9 - 8.0 %) and on day 2 they ranged from 92.9 to 101.7 % (RSD 10.8 - 12.9 %). The method was considered as accurate and precise as all measured values were within the acceptance limits of ICH (2005)[1] and FDA Bioanalytical Method Validation (2001) Guidelines[2].

3 Validation of skin extraction procedure

The ability of the extraction method to recover all of the RA deposited during the in vitro permeation experiments was tested. Porcine skin samples (n=3; area of 0.8 cm^2) were spiked with a known amount of RA in acetone (50, 200 and 1000 ng/cm^2). Acetone facilitated RA skin deposition and was subsequently evaporated. Skin samples were then cut into small pieces and soaked in 2 ml of acetonitrile for 4 h. The skin extracts were then analysed by UHPLC-MS/MS and the amounts of RA recovered were compared to the amounts applied. Results are presented in Table SI3

Table SI3: Validation of RA extraction from skin samples

Applied amount/cm^2 (ng)	Recovered amount/cm^2 (Mean ± SD in ng)	Recovery (Mean ± SD in %)
50	45.5± 4.5	91.1 ± 9.0
200	182.5 ± 5.7	91.3 ± 2.8
1000	916.4± 28.3	91.6 ± 2.8

For all skin samples more than 90% of RA was recovered during the extraction procedure. The extraction method was therefore considered as suitable for RA extraction in the in vitro permeation experiments.

4 DLS supplementary characterization of micelles

The hydrodynamic diameters, polydispersity index, number and volume weighted sizes of micelle formulations is presented in Table SI4.

Table SI4: Micelle size characterization

Formulation	Z_{av} (nm)	P.I.	d_n (nm) [%]dn	d_V (nm) [%]dv
Unloaded micelles	52.3 ± 0.2	0.287 ± 0.019	19.0 ± 2.5 [100.0%]	30.5 ± 2.2 [100.0%]
A	50.5 ± 0.6	0.295 ± 0.008	16.6 ± 0.4 [100.0%]	27.8 ± 0.8 [100.0%]
B	46.1 ± 0.1	0.312 ± 0.009	18.9 ± 0.2 [100.0%]	28.0 ± 0.2 [100.0%]
C	50.4 ± 1.4	0.303 ± 0.011	17.7 ± 2.8 [100.0%]	27.2 ± 2.0 [100.0%]
Formulation A after 6 months of storage at 4°C	31.6 ± 0.2	0.268 ± 0.019	17.4 ± 1.0 [100.0%]	23.8 ± 0.7 [100.0%]
Optimal formulation*	35.8 ± 0.2	0.290 ± 0.030	14.3 ± 2.5 [100.0%]	21.2 ± 2.2 [100.0%]

5 References

1. ICH, in *International Conference on Harmonisation of Technical Requirments for registration of Pharmaceuticals for Human Use*, ed. ICH, 2005.
2. FDA.gov, Guidance for Industry. Bioanalytical Method Validation, 27.05.13