Electronic Supplementary Material (ESI) for Nanoscale.

SUPPLEMENTARY INFORMATION

Selective delivery of adapalene to the human hair follicle under finite dose conditions using polymeric micelle nanocarriers

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1. Validation of HPLC-UV method for quantification of ADA in micelle based formulations

1.1. Specificity

The method was specific for ADA quantitative analysis at 321 nm. ADA was eluted at 7.6 ± 0.1 min. **Figure SI1** presents the chromatograms obtained for blank methanol, TPGS matrix, ADA micelle formulation destruction using methanol and ADA standard. ADA stock solution (100 µg/mL) and all working solutions were prepared using mixture of tetrahydrofuran:methanol (1:1). The volume of injection was 25 µL.

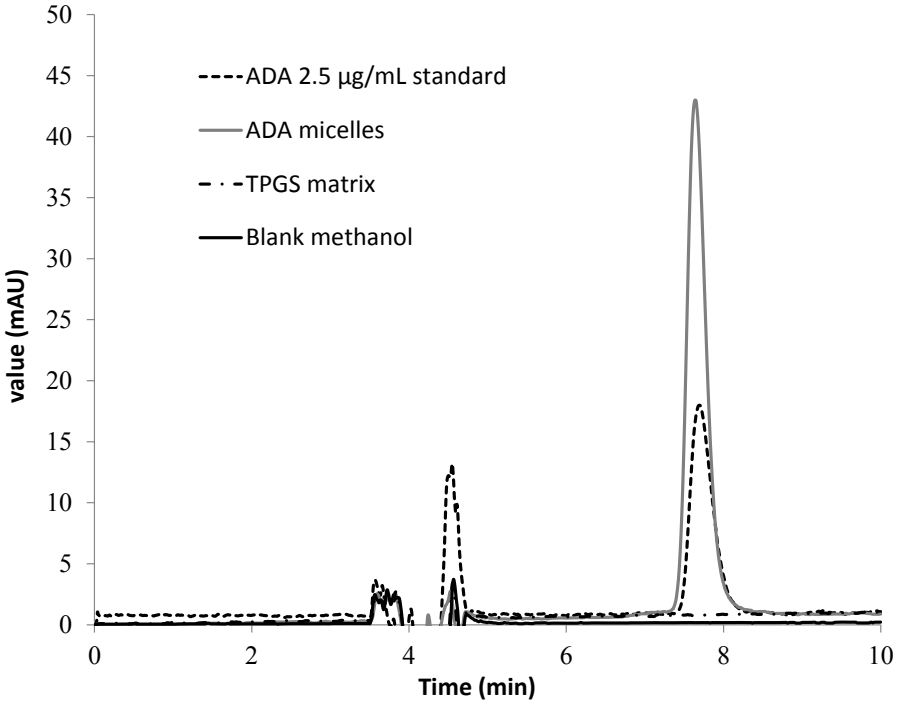

Figure SI1: Chromatograms of blank methanol, TPGS matrix, ADA micelle sample and ADA standard (2.5 µg/mL).

1.2. Limit of detection and limit of quantification

The lower limit of detection (LOD) and lower limit of quantification (LOQ) were determined using the linear regression method and found to be 0.1 µg/mL and 0.3 µg/mL, respectively.

1.3. Linearity

The method was linear in the concentration range of 0.3 - 20 µg/mL with a R^2 of 0.99.

1.4. Accuracy and precision

Intra- and inter-day accuracy and precision was determined using 5.0, 10.0 and 15.0 µg/mL standards. **Table SI1** shows intra- and inter-day accuracy and precision values for ADA quantification method.

Table SI1. Intra- and inter-day accuracy and precision values for ADA quantification method (Mean ± SD).

	Intra-day			Inter-day 1			Inter-day 2		
$[ADA]_{theo}$ (µg/mL)	$[ADA]_{meas}$[a] (µg/mL)	RSD (%)	Recovery (%)	$[ADA]_{meas}$[a] (µg/mL)	RSD (%)	Recovery (%)	$[ADA]_{meas}$[a] (µg/mL)	RSD (%)	Recovery (%)
5	5.05 ± 0.04	0.72	101.0	5.1 ± 0.02	0.39	102.1	5.07 ± 0.01	0.24	101.4
10	10.11 ± 0.02	0.24	101.1	10.18 ± 0.03	0.30	101.8	10.11 ± 0.05	0.46	101.1
15	14.82 ± 0.01	0.04	98.8	14.96 ± 0.01	0.07	99.8	14.83 ± 0.05	0.33	98.9

The method was considered as accurate and precise as all measured values presented in Table SI1 were within the acceptance limits of validation guidelines [1, 2].

2. Validation of UHPLC-MS/MS method for quantification of ADA present in the skin samples

ADA detection was performed using UHPLC-MS/MS. The gradient chromatographic elution method used for ADA is provided in **Table SI2**. The mass spectrometer settings are provided in **Table SI3**.

2.1 Specificity

The method was considered to be specific for ADA quantitative using MRM transition monitored for 411.104→367.1 ADA was eluted at 1.24 min. Figure SI2 shows the chromatogram of a) blank and b) ADA standard in skin extract.

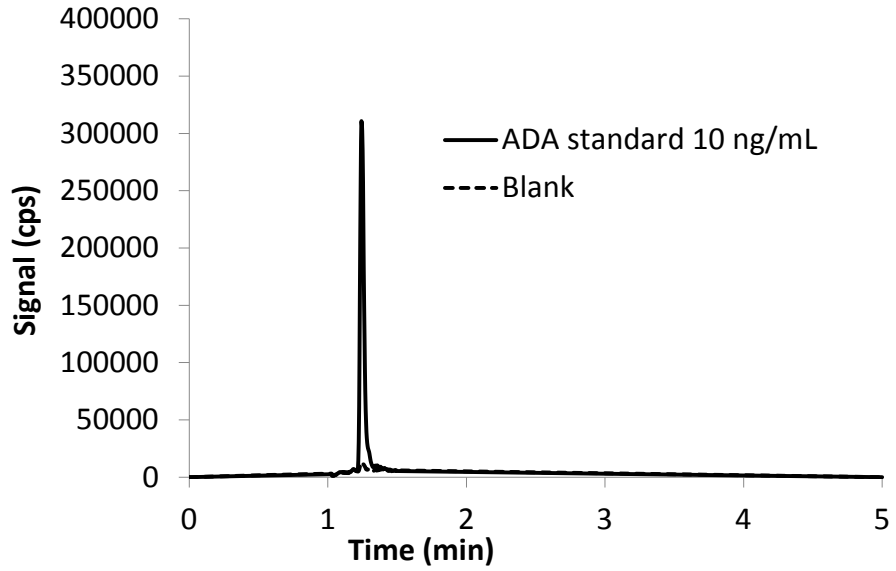

Figure SI2: Chromatograms of ADA standard (10 ng/mL), and blank skin extract.

Table SI2. Gradient chromatographic elution method used for ADA

Time (min)	Flow rate(mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.0	0.2	75.0	25.0
0.5	0.2	35.0	65.0
1.5	0.2	35.0	65.0
2.0	0.2	10.0	90.0
2.5	0.2	10.0	90.0
3.0	0.2	75.0	25.0
5.0	0.2	75.0	25.0

2.2. Limit of detection and limit of quantification

The lower limit of detection (LOD) and lower limit of quantification (LOQ) were determined using the linear regression equation and found to be 1.0 ng/mL and 3.0 ng/mL, respectively.

2.3. Linearity

The method was found to be linear in the concentration range of 3-200 ng/mL ($R^2 = 0.99$) for standards prepared in porcine and human skin extract. The porcine or human skin extract was prepared by soaking one cm^2 skin in 5 mL of tetrahydrofuran:methanol (1:1) mixture. The

linearity in phosphate buffer saline containing 1% Tween-80 was in the concentration range of 5-50 ng/mL ($R^2 = 0.99$).

Table SI3. MS/MS settings for detection of ADA

Parameters	Values
Nature of parent ion	$[M - H]^-$
Parent ion (m/z)	411.104
Daughter ion (m/z)	367.1
MS mode collision energy (V)	3.0
MS/MS mode collision energy (V)	23.0
Cone voltage (V)	10
Capillary voltage (kV)	3.40
Source temperature (°C)	150
Desolvation temperature (°C)	500
Desolvation gas flow (L h^{-1})	650
Cone gas flow (L h^{-1})	0
LM resolution 1	9.41
HM resolution 1	14.90
Ion energy 1 (V)	0.20
LM resolution 2	9.69
HM resolution 2	14.85
Ion energy 2 (V)	0.90

2.4. Accuracy and precision

Intra- and inter-day accuracy and precision was determined using 10, 50 and 150 ng/mL standards. **Table SI4** shows intra- and inter-day accuracy and precision values for ADA quantification method.

Table SI4. Intra- and inter-day accuracy and precision values for ADA quantification method (Mean ± SD).

	Intra-day			Inter-day 1		
$[ADA]_{theo}$ (ng/mL)	$[ADA]_{meas}$[a] (ng/mL)	RSD (%)	Recovery (%)	$[ADA]_{meas}$[a] (ng/mL)	RSD (%)	Recovery (%)
10	10.47±0.06	0.55	104.7	11.19±0.12	1.1	111.94
50	50.61±0.84	1.67	101.22	53.10±0.67	1.26	106.1
150	146.42±0.91	0.62	97.62	154.07±1.67	1.1	102.71

The method was considered as accurate and precise as all measured values presented in **Table SI4** were within the acceptance limits of validation guidelines [1, 2].

3. Effect of TPGS copolymer concentration on ADA content

The effect of TPGS copolymer concentration on ADA loading, ADA content and incorporation efficiency in the micelle formulations was investigated by using different copolymer concentrations (i.e. 10, 25, 50, 75, 100, 150 mg/mL) while keeping the ADA concentration fixed at 0.25 mg/mL.

Table SI5. Effect of TPGS copolymer concentration on ADA content, ADA loading and incorporation efficiency (Mean ± SD).

			ADA content	
Copolymer content (mg/mL)	**ADA:Copolymer ratio**	**ADA loading (mg_{ADA}/g_{copol})**	**ADA content ($mg_{ADA}/mL_{formulation}$)**	**Incorporation efficiency (%)**
10	1:40	1.33 ± 0.17	0.013 ± 0.001	5.33 ± 0.68
25	1:100	1.27 ± 0.10	0.031 ± 0.002	12.74 ± 1.00
50	1:200	1.54 ± 0.11	0.077 ± 0.006	30.84 ± 2.21
75	1:300	1.57 ± 0.06	0.116 ± 0.004	46.30 ± 1.55
100	1:400	1.81 ± 0.07	0.182 ± 0.006	72.69 ± 2.41
150	1:600	1.45 ± 0.08	0.217 ± 0.006	87.00 ± 2.31

4. Validation of ADA skin extraction procedure

The ability of the skin extraction method to recover maximum amount of ADA deposited in the skin after *in vitro* cutaneous delivery experiments was tested. Porcine skin samples (n = 3; area of 1.13 cm^2) were chopped into small pieces and spiked with a known amount of ADA in acetone (1.0 and 2.5 µg/cm^2). Acetone enabled ADA skin deposition and was subsequently gets evaporated. Skin samples were then soaked in 5.65 ml of tetrahydrofuran:methanol (1:1) for 24 h. The skin extraction samples were centrifuged, filtered and analyzed by validated UHPLC-MS/MS and the amounts of ADA recovered were compared to the amounts applied. Results are presented in **Table SI5**.

Table SI6. Validation of ADA extraction recovery from skin samples (Mean ± SD).

Amount of ADA applied (µg/cm^2)	Amount of ADA recovered (µg/cm^2)	Recovery (%)
1	0.93 ± 0.03	93.31 ± 3.03
2.5	2.23 ± 0.07	90.0 ± 2.67

From all skin samples >= 90% of ADA was recovered after extraction procedure. The extraction method was therefore considered as suitable for ADA extraction from the skin samples.

5. Settings used for confocal laser scanning microscope

For the acquisition of CLSM images the objective used was EC Epiplan 10x/0.2 M27. The filters used were 396-477 nm. Pinhole and master gain values were 519 µm 952, respectively. Adapalene was excited by multiphoton laser at 720 nm.

6. References.

1. International Conference on Harmonisation, Validation of Analytical Procedures: Text and Methodology Topic Q 2 (R1), 2005.

2. FDA. Guidance for Industry: Bioanalytical Method Validation, 2001.