Supporting Information

Enhancing skin delivery of tranexamic acid via esterification: synthesis and evaluation of alkyl ester derivatives

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HPLC analysis

TA and its derivatives were quantified using a Hitachi L2000 high-performance liquid chromatography (HPLC) system (Hitachi, Tokyo, Japan), fitted with a Diamonsil® C18 column (250 mm × 4.6 mm, 5 μm; Dikma Technologies, Beijing, China). The column temperature was controlled at 30°C. A flow rate of 0.5 mL/min was maintained throughout the analysis. Detection was carried out using a UV detector set at a wavelength of 220 nm. The mobile phase compositions varied according to the sample type: for TA, the mobile phase was a mixture of 20% methanol and 80% 0.01 M KH_2PO_4 solution; for TA4, it comprised 70% methanol and 30% 0.01 M KH_2PO_4 solution; and for TA8, it consisted of 65% acetonitrile and 35% 0.01 M KH_2PO_4 solution. Each sample had an injection volume of 20 μL.

LC-MS/MS analysis

Following initial sample pre-treatment, the analytes were subjected to LC-MS/MS analysis. An Agilent 1260 Infinity LC system (Agilent Technologies, Santa Clara, CA, USA) was utilized, coupled with an API 4000 triple quadrupole MS/MS system (AB Sciex, Framingham, MA, USA). A C18 analytical column (50 mm × 2.0 mm, 4 μm; Synergi 4u Hydro-RP, Phenomenex, Torrance, CA, USA) was used and protected with a C18 guard column (4 mm × 2.0 mm, 3 μm; SecurityGuard, Phenomenex, Torrance, CA, USA). A gradient elution method was employed for the mobile phase, consisting of solvent A: 5 mM ammonium acetate with 0.1% formic acid, and solvent B: methanol with 0.1% formic acid. The gradient elution protocol was as follows: 0 ~ 0.6 min, 98% A; 0.6~1.0 min, 98% A to 15% A; 1.0~2.0 min, 15% A to 5% A; 2.0~3.0 min, 5% A; 3.0~3.1 min, 5% A to 98% A; 3.1~5.5 min, 98% A. The flow rate was maintained at 0.4 mL/min, the column temperature was set to 40°C, and the injection volume was 10 μL. The MS detection utilized multiple reaction monitoring (MRM) in positive ion mode, with electrospray ionization (ESI) as the ionization source. Key operational parameters were set to an ion spray voltage of 4500 V and an ion source temperature of 500°C.

The monitored ion pairs for quantification were: TA with m/z transitions from 158.1 to 95.2, TA4 from 214.0 to 95.2, TA8 from 270.1 to 95.2, and diphenhydramine from 256.3 to 166.9. Gas parameters included 40 psi for GS1 (nebulizer gas, nitrogen), 50 psi for GS2 (drying gas, nitrogen), 20 psi for CUR (curtain gas, nitrogen), and 12 psi for CAD (collision gas, nitrogen). Specific parameters such as declustering potentials (DP), collision energies (CE), entrance potentials (EP), and collision exit potentials (CXP) were optimized for each ion transition.

Water loss measurement of gels

To evaluate the water loss rate in TA8 gel formulations, samples with varying propylene glycol concentrations (0%, 5%, 10%, and 15%) were prepared. Each formulation maintained a constant quantity of other excipients. Precisely 20 g of each gel sample were placed in identical glass Petri dishes. These dishes were then subjected to controlled drying conditions in an oven set at 45°C, with the Petri dish lids left open to facilitate evaporation. The dehydration process was monitored by weighing the dishes at one-hour intervals. The weight measurements at each time point enabled the calculation of the dehydration rate, providing insights into the influence of propylene glycol concentration on the water loss behavior of the gel formulations.

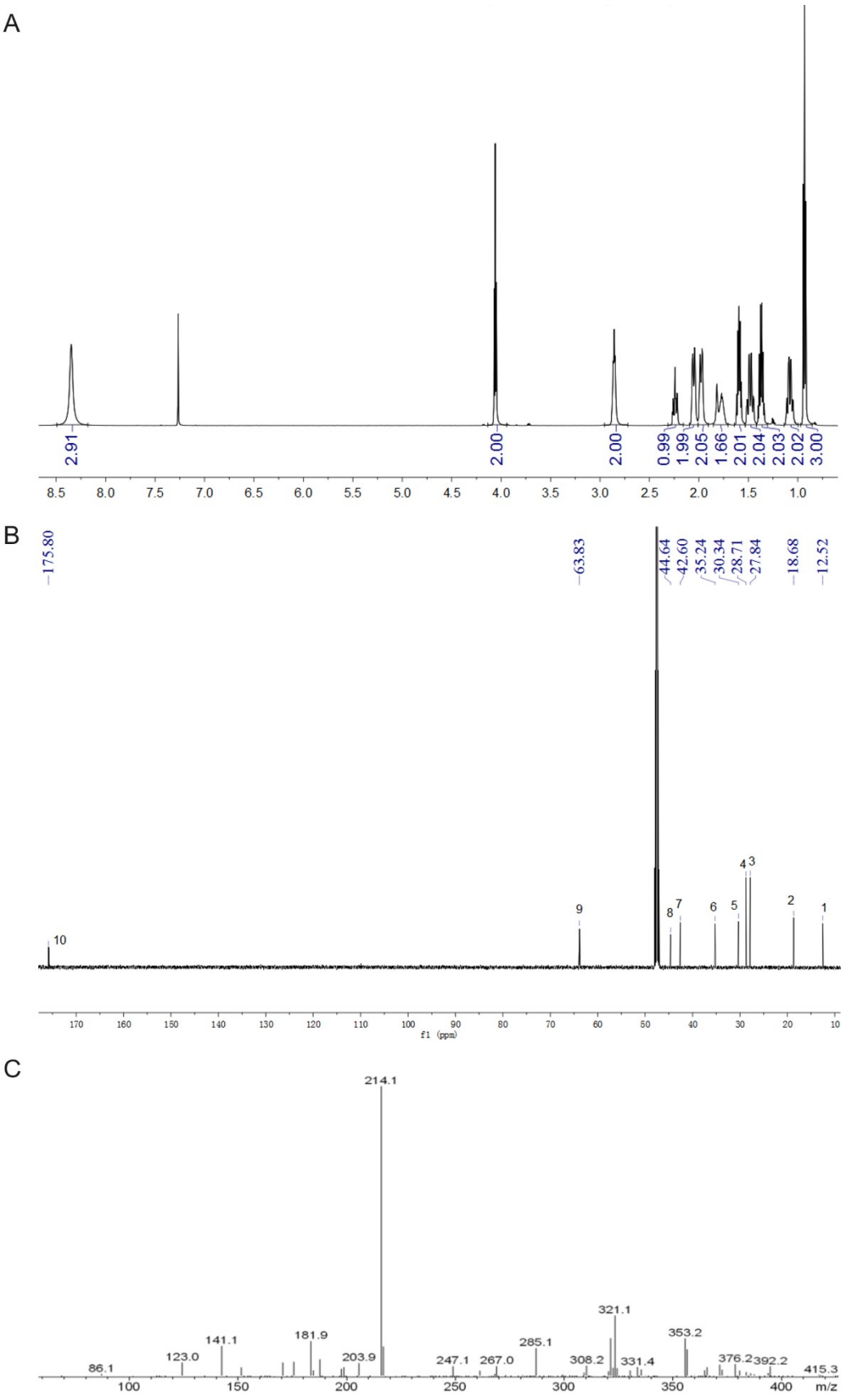

Figure S1. NMR and mass spectra of TA4. (A) ^{1}H-NMR spectrum of TA4 in $CDCl_3$. (B) ^{13}C-NMR spectrum of TA4 in MeOD. (C) Mass spectrum of TA4.

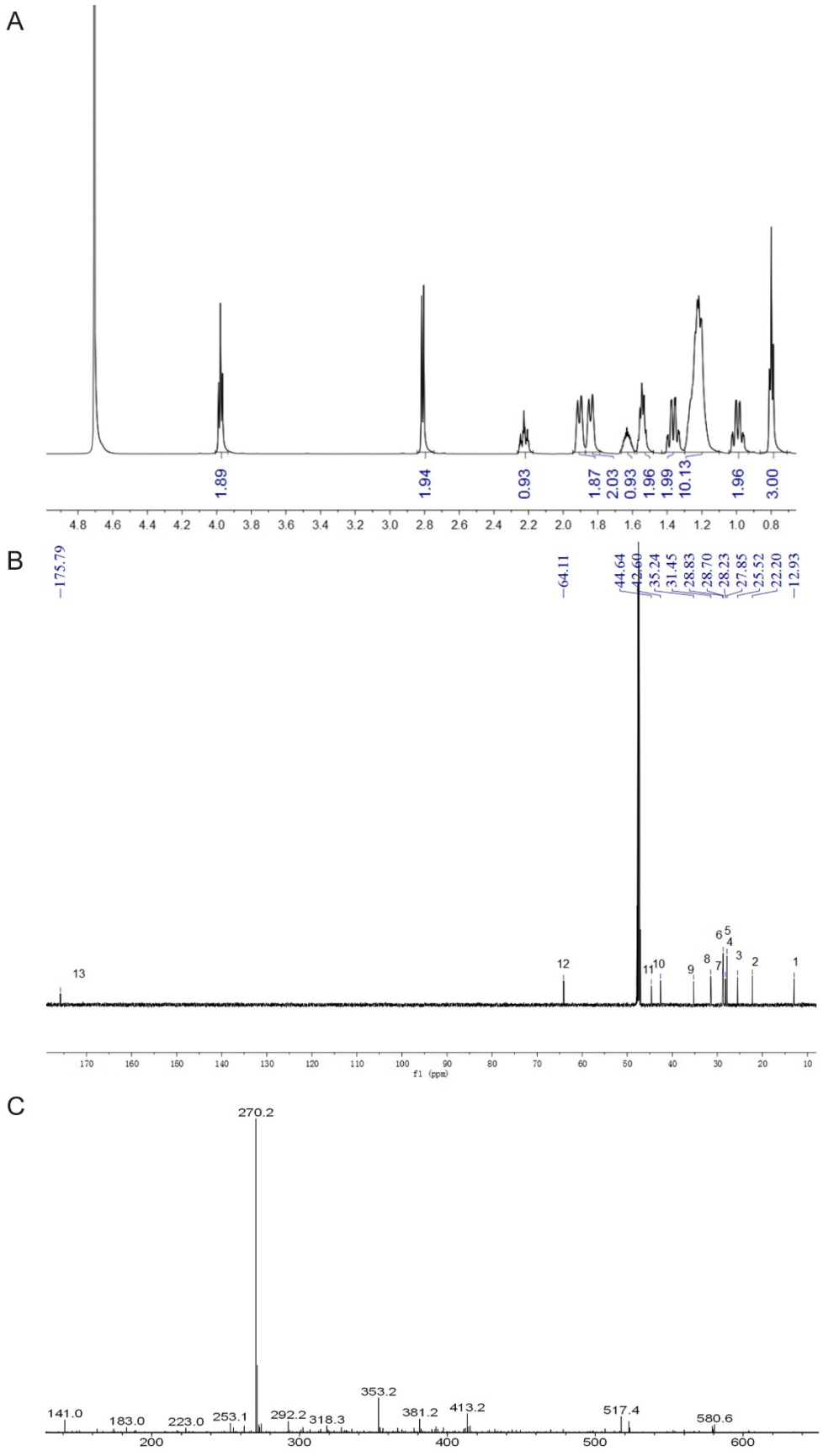

Figure S2. NMR and mass spectra of TA8: (A) ^{1}H-NMR spectrum of TA8 in $CDCl_3$. (B) ^{13}C-NMR spectrum of TA8 in MeOD. (C) Mass spectrum of TA8.

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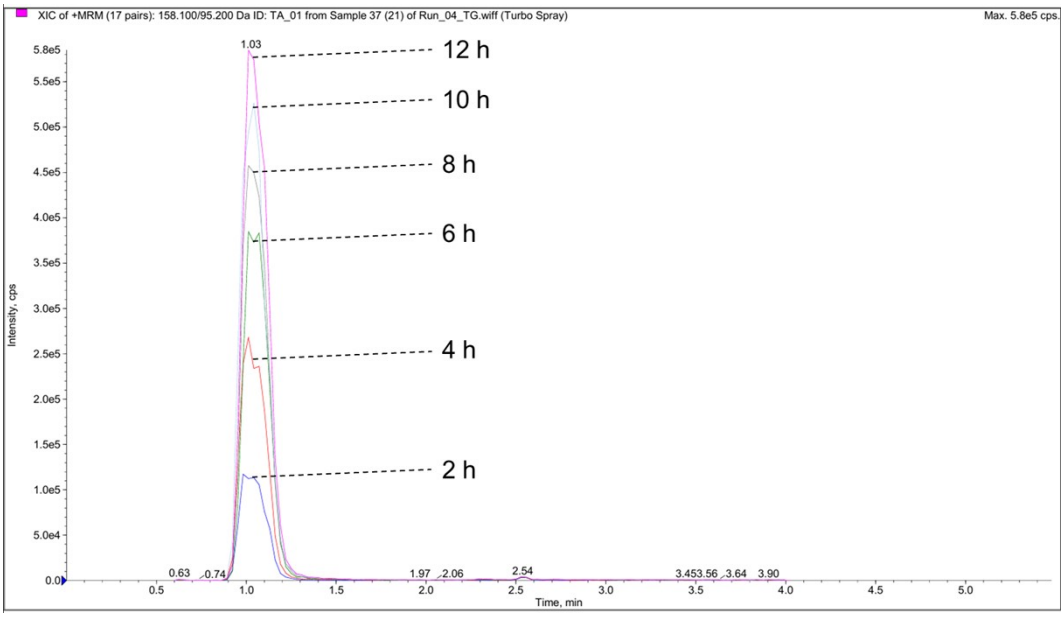

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65 Figure S3. Representative extracted ion chromatograms (XICs) of TA (*m/z* 158.1 → 95.2) in

66 receptor solution from the skin permeation study following TA solution application.

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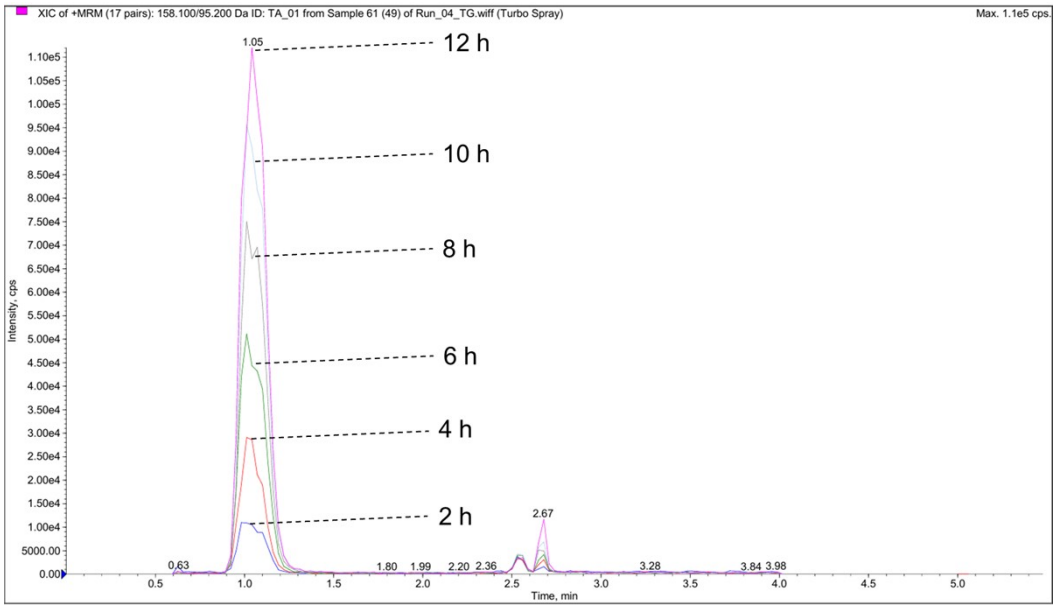
A



B

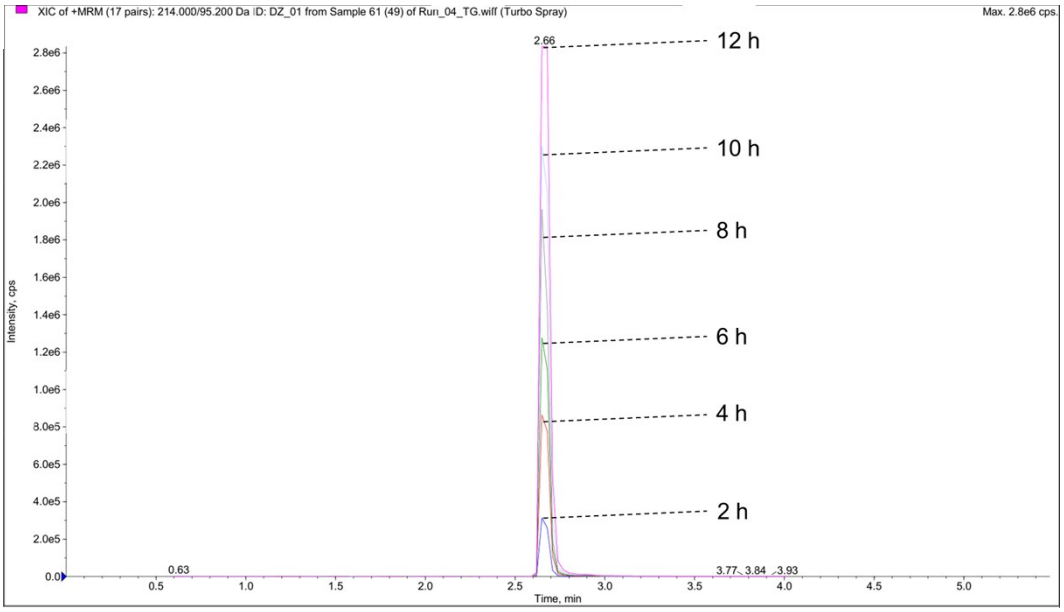
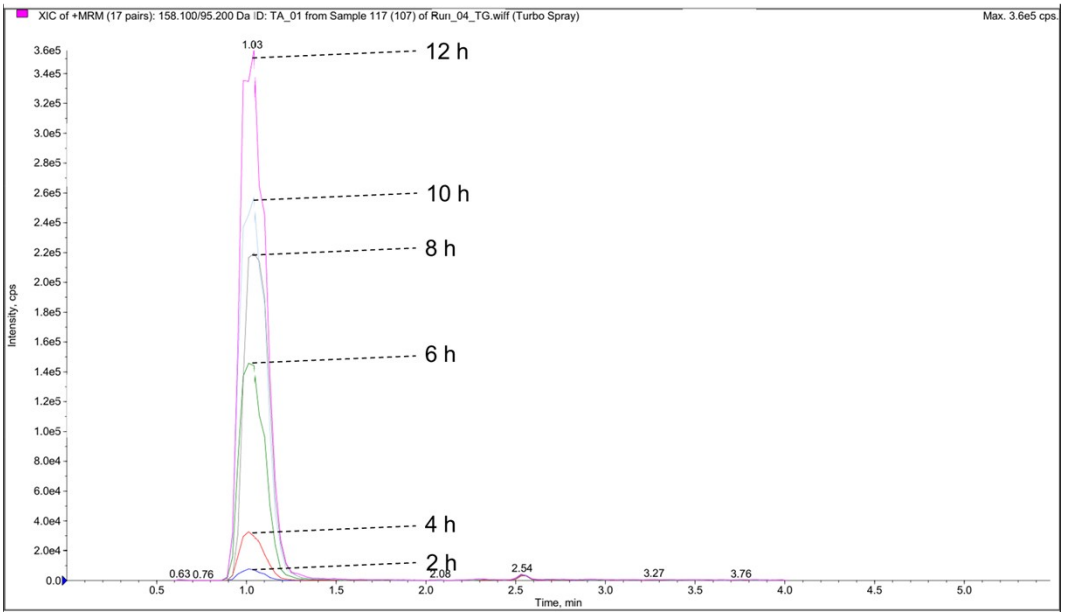


Figure S4. Representative extracted ion chromatograms (XICs) of (A) TA (*m/z* 158.1 → 95.2) and (B) TA4 (*m/z* 214.2 → 95.2) in receptor solution from the skin permeation study following TA4 solution application.

A



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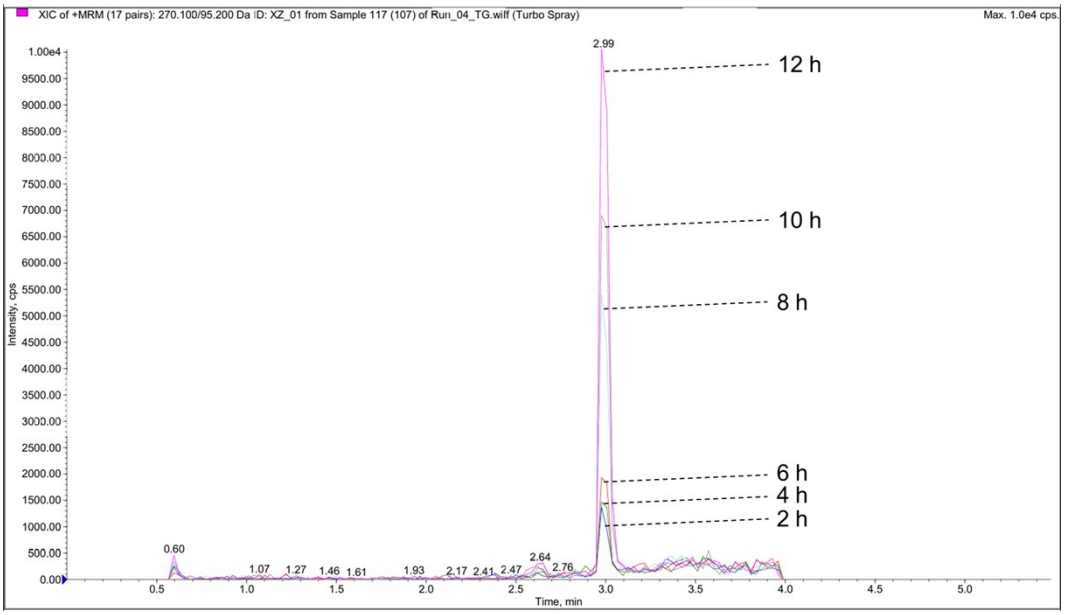

Figure S5. Representative extracted ion chromatograms (XICs) of (A) TA (*m/z* 158.1 → 95.2) and (B) TA8 (*m/z* 270.1 → 95.2) in receptor solution from the skin permeation study following TA8 solution application.

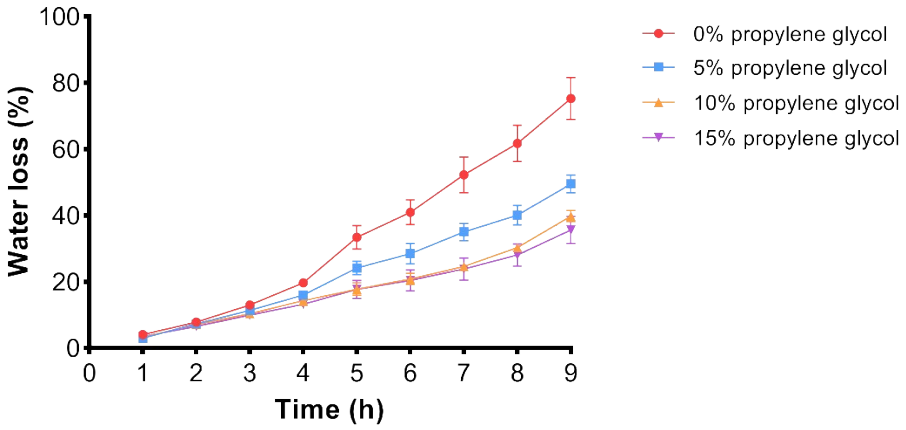

Figure S6. Water loss of TA8 gels with different concentrations of propylene glycol over time.

Table S1. Cumulative amounts of TA, TA4 and TA8 permeated through porcine skin over time (n = 4).

t (h)	Q_n (nmol/cm^2)				
	TA group	TA4 group		TA8 group	
	TA	TA4	TA	TA8	TA
2	18.65 ± 2.04	8.31 ± 0.57	0.00	15.05 ± 4.00	8.90 ± 1.28
4	37.16 ± 3.01	31.95 ± 1.03	0.00	41.32 ± 4.84	25.49 ± 2.00
6	56.22 ± 0.56	66.91 ± 6.79	0.00	64.41 ± 6.43	48.84 ± 2.49
8	72.66 ± 10.95	105.40 ± 4.87	0.07 ± 0.04	85.01 ± 10.24	72.12 ± 6.98
10	90.70 ± 14.95	140.61 ± 4.09	0.09 ± 0.02	120.61 ± 14.49	101.79 ± 7.46
12	106.37 ± 15.20	200.25 ± 12.53	0.13 ± 0.07	166.84 ± 2.97	139.74 ± 13.75