Synergistic Beeswax-Based Nano-formulation for Enhanced Chronic Wound Healing and Antibacterial Potency

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Table S1: Forced Degradation Profile of Individual APIs and Formulation F4.

Stress Condition	% Degradation, SHA	% Degradation, LIDO	% Degradation, DLX	% Degradation, F4 (Total)	Major Degradation Products Identified (Retention Time, min)
Acidic Hydrolysis (0.1 N HCl, 6h)	2.1 ± 0.5	6.5 ± 1.0	**18.5 ± 2.0**	**5.1 ± 0.9**	**LIDO** degradant at 0.93 min (N-dealkylation); **DLX** degradants at 1.89 min (defluorination) and 3.97 min.
Alkaline Hydrolysis (0.1 N NaOH, 6h)	3.8 ± 0.7	8.2 ± 1.2	**19.8 ± 2.1**	**6.2 ± 1.1**	**DLX** degradant at 1.71 min (ring opening); **LIDO** degradant at 7.90 min (amide hydrolysis).
Oxidative Degradation (3% H_2O_2, 6h)	3.1 ± 0.6	7.9 ± 1.1	**17.6 ± 1.9**	**5.8 ± 1.0**	**DLX** degradant at 4.29 min (quinone-imine formation); **LIDO** degradant at 13.18 min (N-oxide).
Thermal Degradation (60°C, 5 days)	3.8 ± 0.7	6.2 ± 1.3	**11.4 ± 1.8**	**4.1 ± 0.9**	No significant new degradation peaks detected.
Photolytic Degradation (2 days)	1.5 ± 0.4	6.3 ± 1.0	**19.2 ± 2.2**	**5.9 ± 1.2**	**DLX** degradant at 13.75 min (photoisomerization).
Nitrosating Condition (0.01% $NaNO_2$, pH 3.0, 37°C, 18 h)	1.2 ± 0.3	**5.8 ± 0.9**	7.1 ± 1.2	**2.9 ± 0.6**	**LIDO** degradant at 6.37 min (potential nitrosamine N1); **2.5% in free LIDO vs. <0.5% in F4**.

Table S2. ICH M7(R2) Assessment Summary

Impurity	Toxtree	ProTox-3	ICH M7 Class	Level (F4)	TTC Limit	Control
DLX Defluorination	–ve	–ve	5	<0.5%	≤1.5 µg/day	HPLC
LIDO N-Dealkylation	–ve	–ve	5	<1%	≤1.5 µg/day	HPLC
Nitrosamine (LIDO-derived)	–ve	–ve	5	<0.5%	≤18 ng/day	Encapsulation

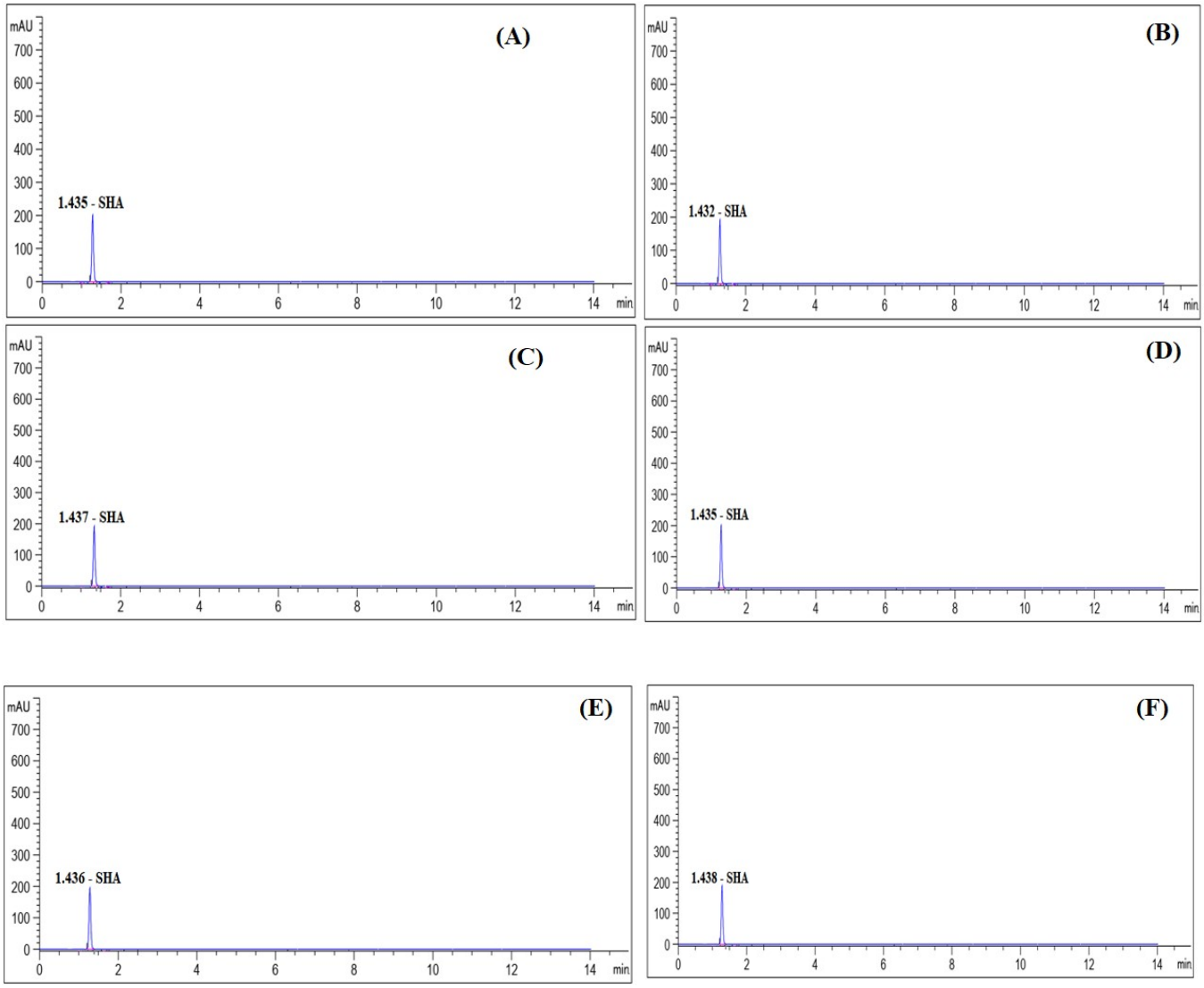
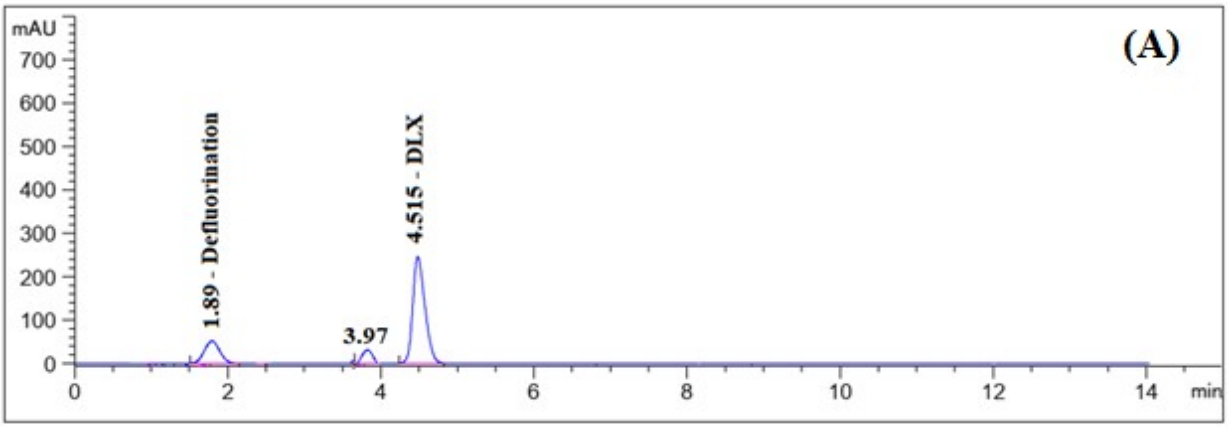
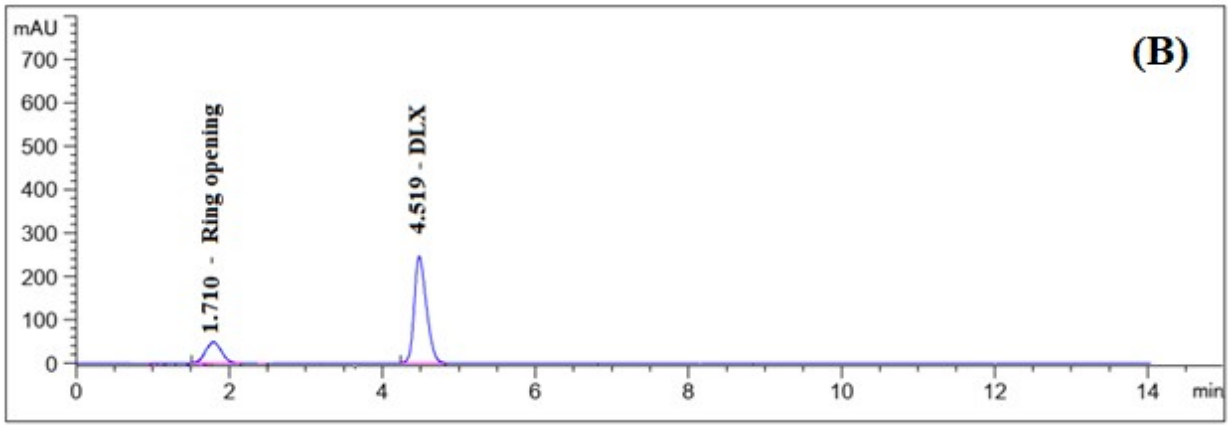
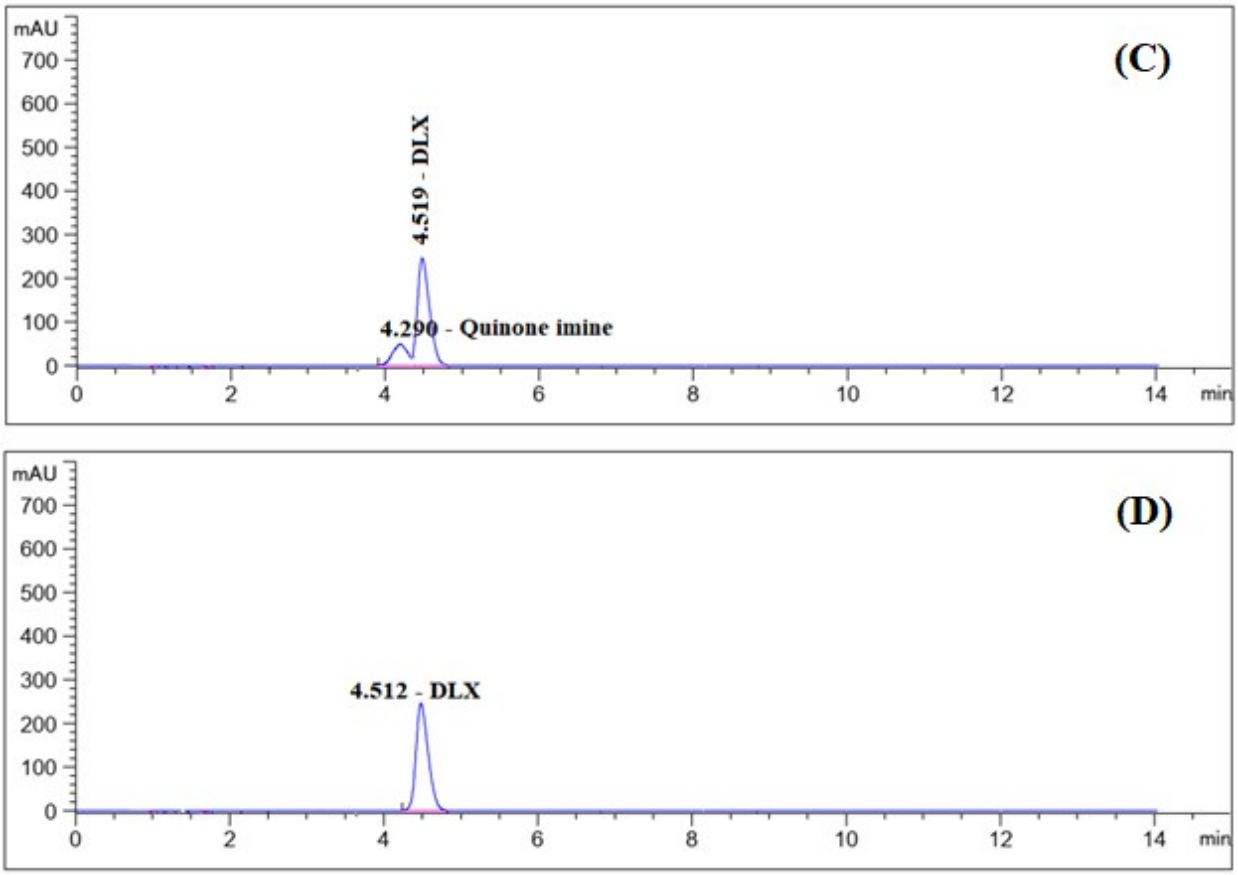


Figure S1. HPLC Chromatograms of Forced Degradation Studies for sodium hyaluronate (SHA), including (A) acidic hydrolysis (6 h), (B) alkaline hydrolysis (6 h), (C) oxidative stress (6 h), (D) thermal stress (5 days), (E) photolytic stress (2 days), and (F) nitrosating conditions (18 h).


(A)
mAU
700
600
500
400
300
200
100
0
1.89 - Defluorination
3.97
4.515 - DLX
0
2
4
6
8
10
12
14
min


(B)
mAU
700
600
500
400
300
200
100
0
1.710 - Ring opening
4.519 - DLX
0
2
4
6
8
10
12
14
min


(C)
mAU
700
600
500
400
300
200
100
0
4.519 - DLX
4.290 - Quinone imine
0
2
4
6
8
10
12
14
min
(D)
mAU
700
600
500
400
300
200
100
0
4.512 - DLX
0
2
4
6
8
10
12
14
min

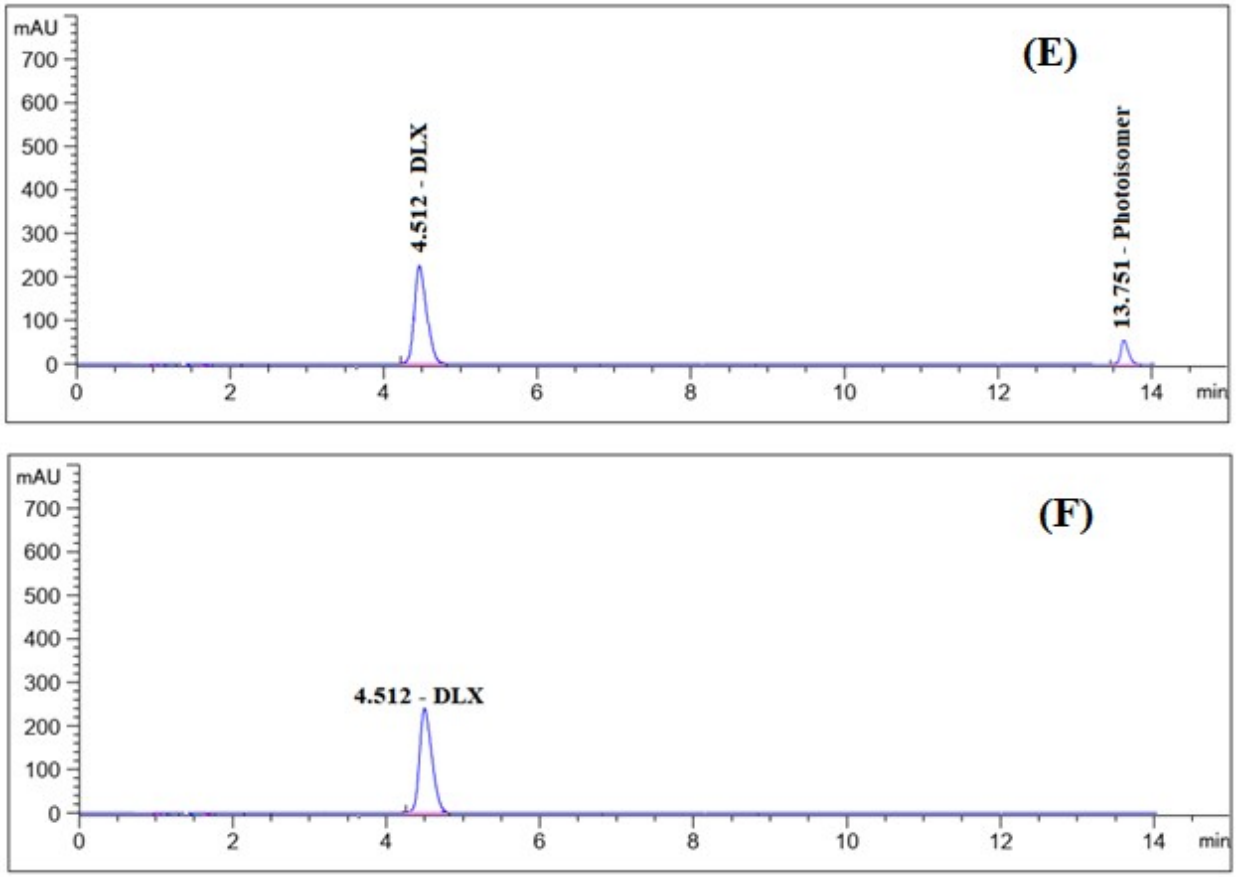
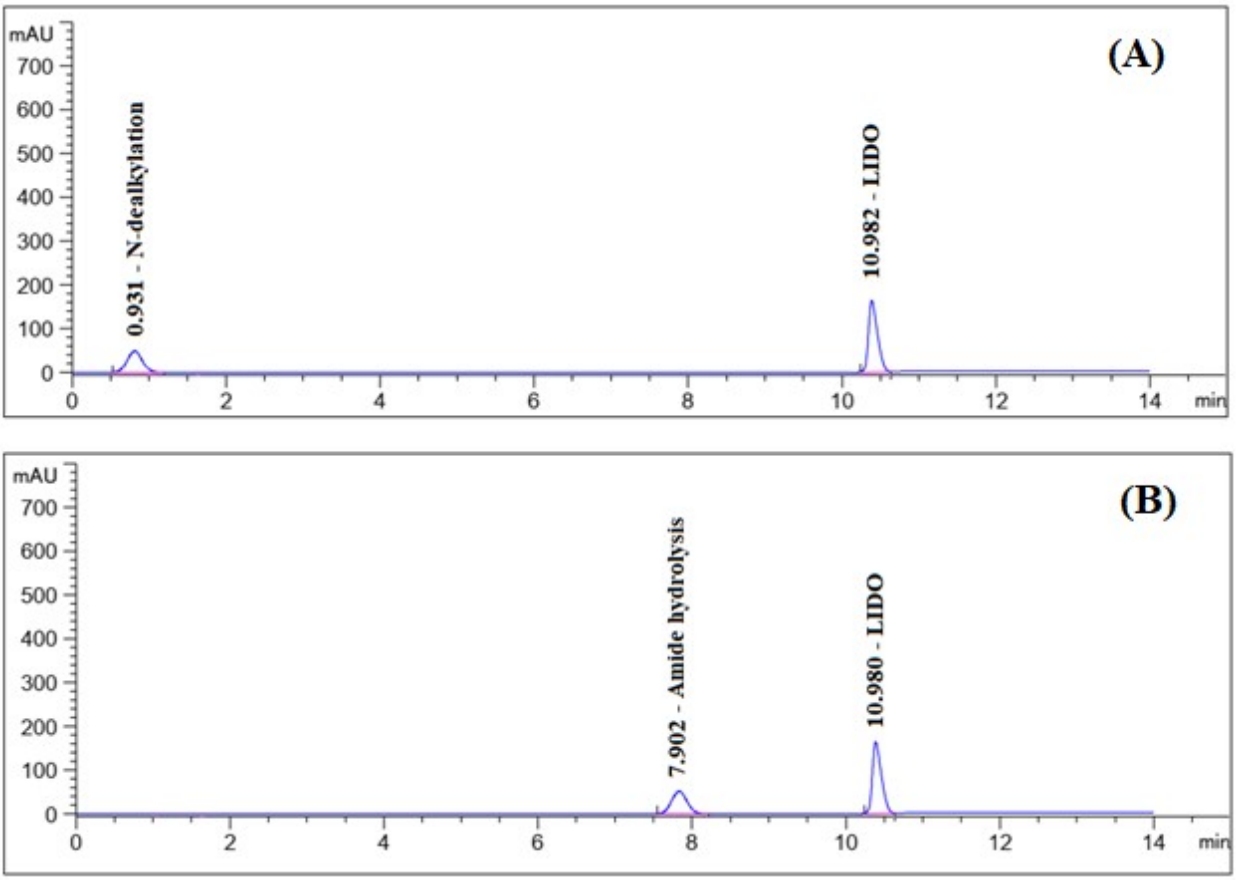
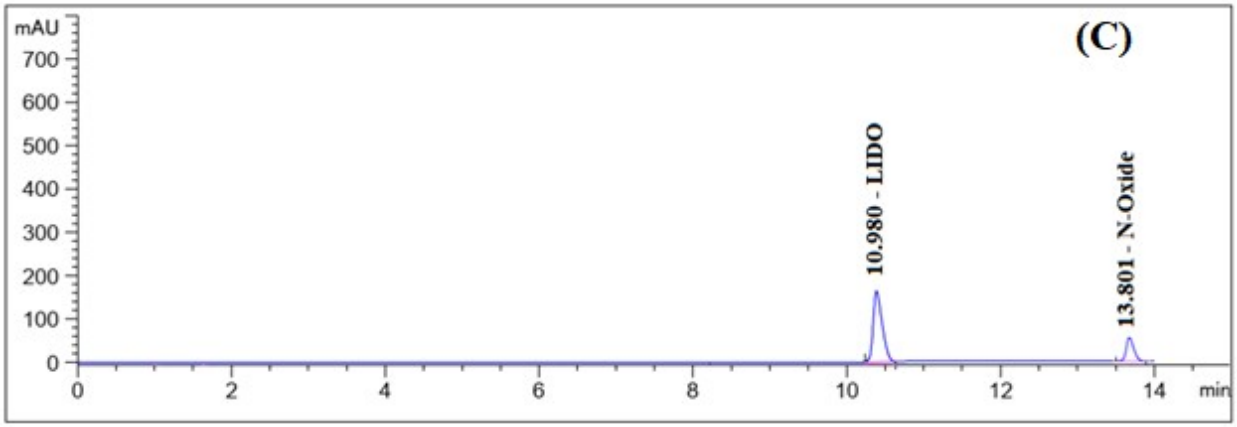
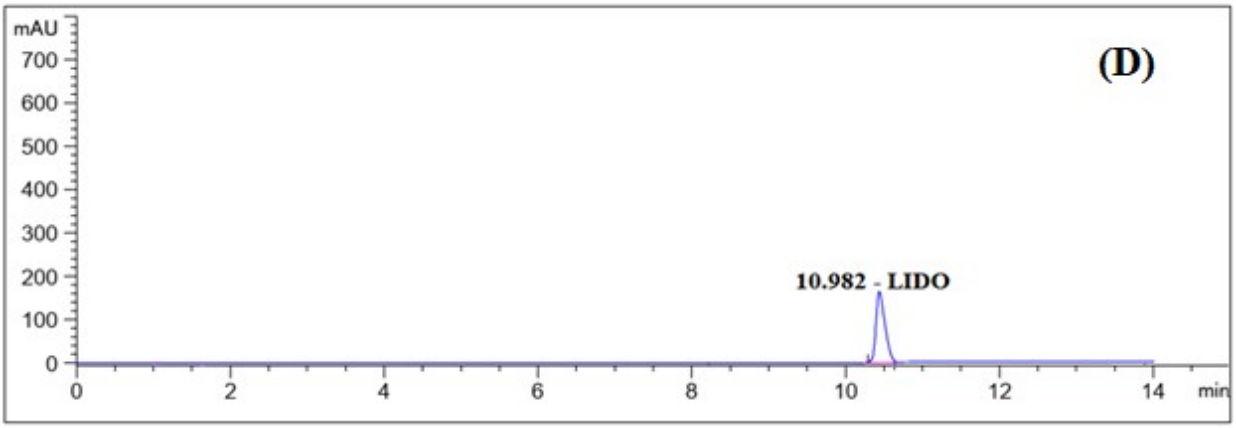


Figure S2. HPLC Chromatograms of Forced Degradation Studies for Delafloxacin (DLX), including (A) acidic hydrolysis (6 h), (B) alkaline hydrolysis (6 h), (C) oxidative stress (6 h), (D) thermal stress (5 days), (E) photolytic stress (2 days), and (F) nitrosating conditions (18 h).


(A)
mAU
700
600
500
400
300
200
100
0
0.931 - N-dealkylation
10.982 - LIDO
0
2
4
6
8
10
12
14
min
(B)
mAU
700
600
500
400
300
200
100
0
7.902 - Amide hydrolysis
10.980 - LIDO
0
2
4
6
8
10
12
14
min


(C)
mAU
700
600
500
400
300
200
100
0
10.980 - LIDO
13.801 - N-Oxide
0
2
4
6
8
10
12
14
min


(D)
mAU
700
600
500
400
300
200
100
0
10.982 - LIDO
0
2
4
6
8
10
12
14
min

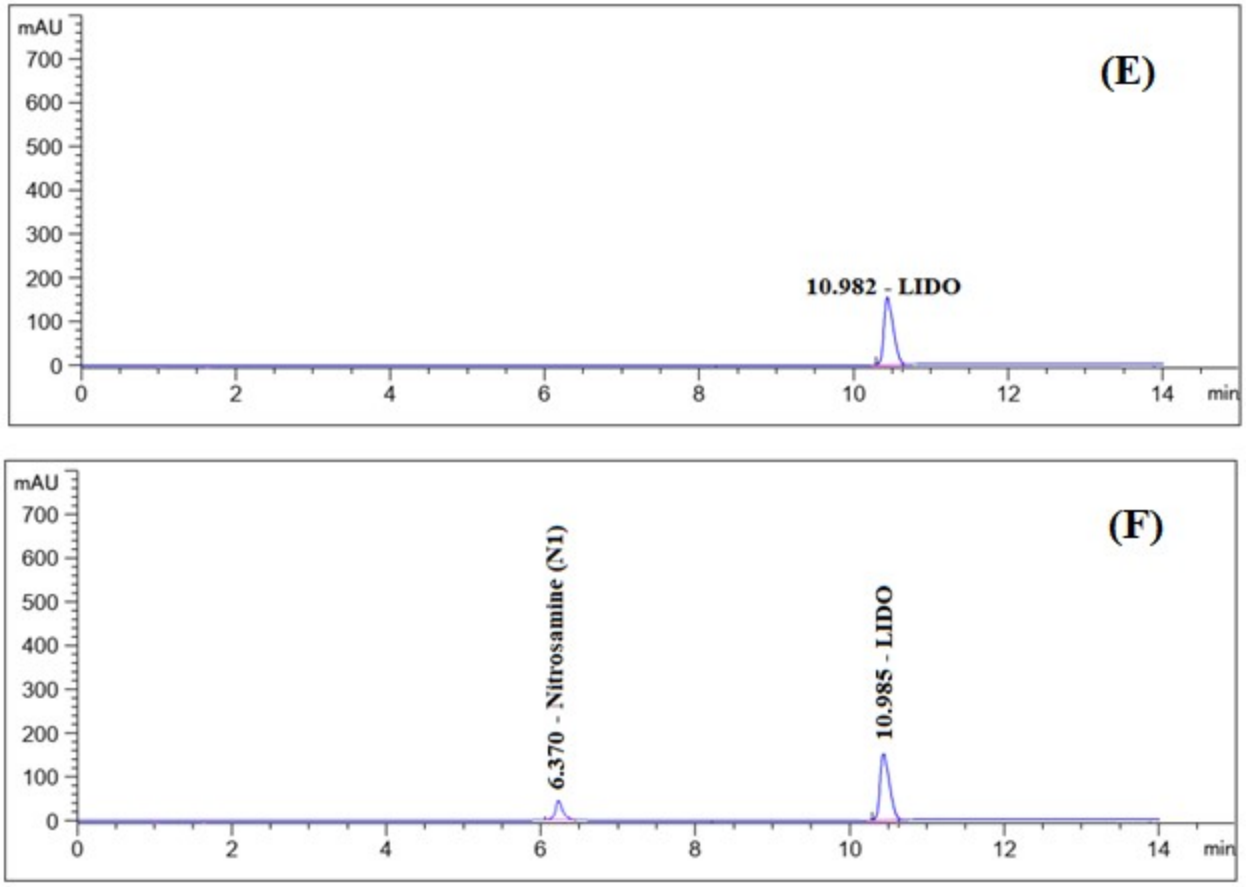
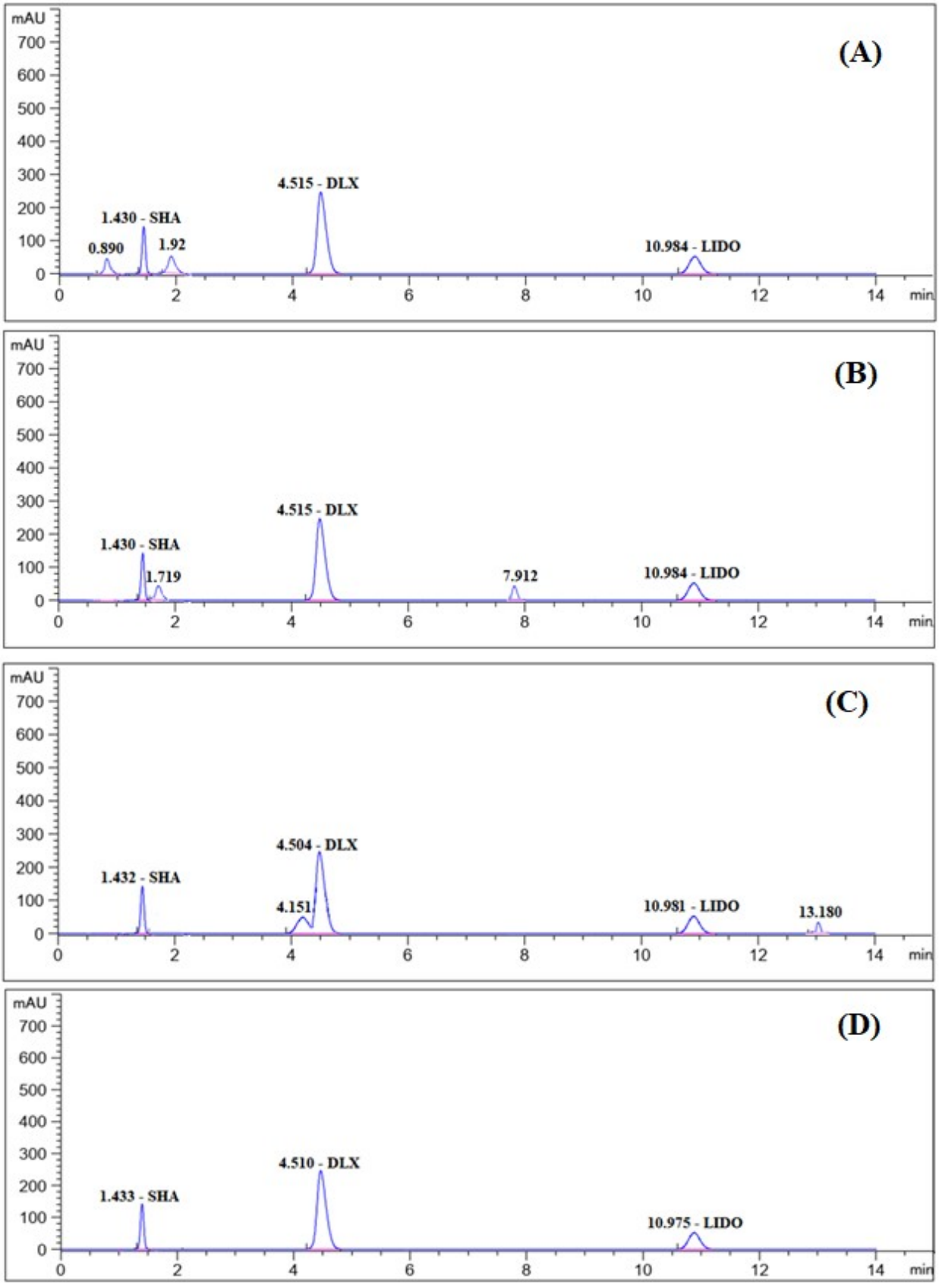


Figure S3. HPLC Chromatograms of Forced Degradation Studies for Lidocaine HCL (LIDO), including (A) acidic hydrolysis (6 h), (B) alkaline hydrolysis (6 h), (C) oxidative stress (6 h), (D) thermal stress (5 days), (E) photolytic stress (2 days), and (F) nitrosating conditions (18 h).


(A)
mAU
0.890
1.430 - SHA
1.92
4.515 - DLX
10.984 - LIDO
min
(B)
1.430 - SHA
1.719
4.515 - DLX
7.912
10.984 - LIDO
(C)
1.432 - SHA
4.151
4.504 - DLX
10.981 - LIDO
13.180
(D)
1.433 - SHA
4.510 - DLX
10.975 - LIDO

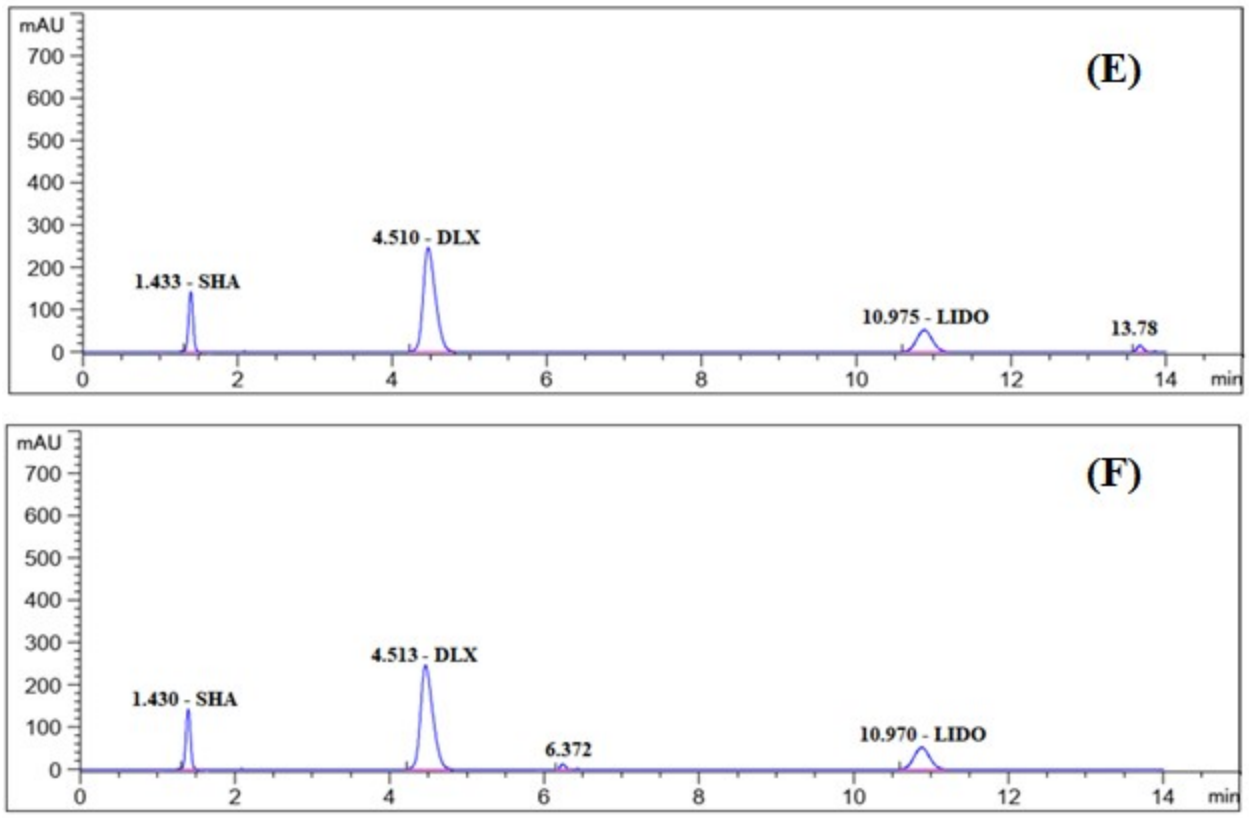


Figure S4. HPLC Chromatograms of Forced Degradation Studies for F4 formulation including (A) acidic hydrolysis (6 h), (B) alkaline hydrolysis (6 h), (C) oxidative stress (6 h), (D) thermal stress (5 days), (E) photolytic stress (2 days), and (F) nitrosating conditions (18 h)