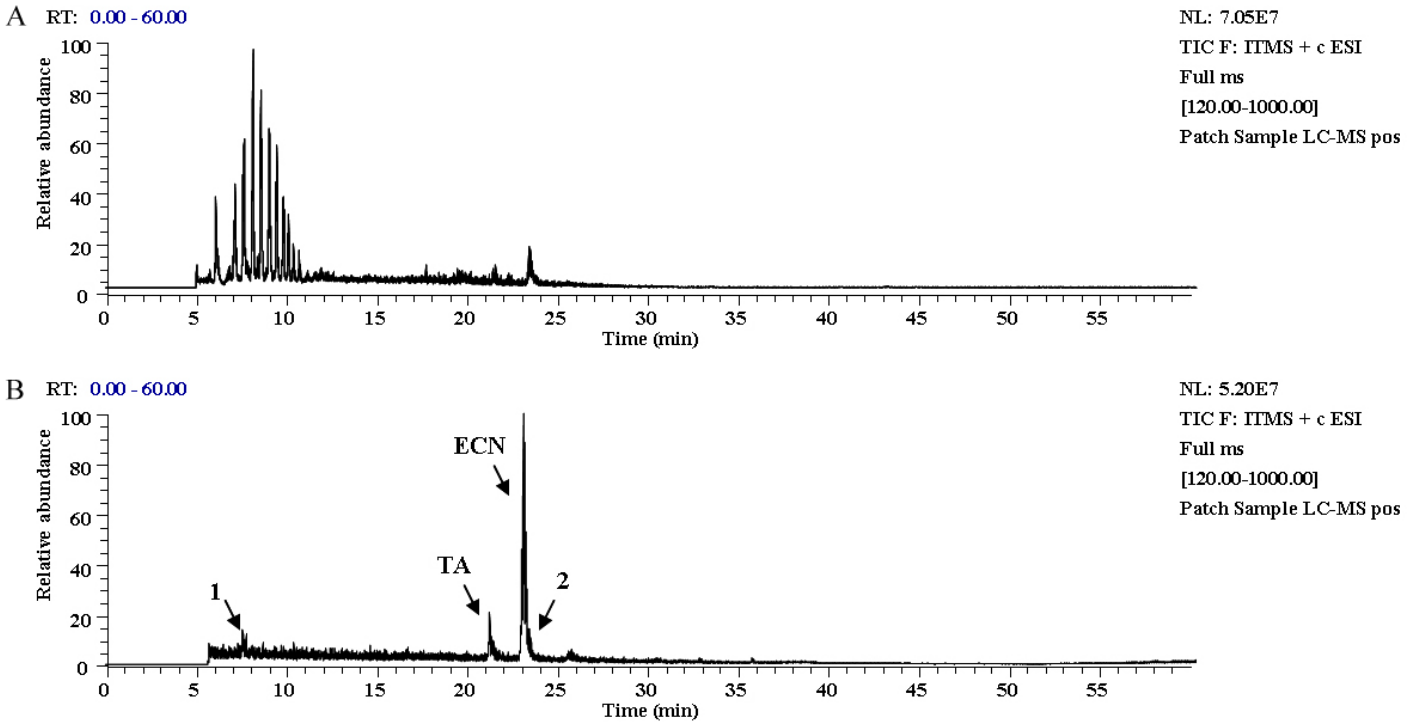
Supplementary Material

This supplementary content contains the purification of tape samples via solid-phase extraction (SPE), the identification of the structure of the synthesized oxidative product of econazole nitrate (designated ECN-O), and the method validation for the quantitative analysis of ECN-O by high-performance liquid chromatography (HPLC).

1 PURIFICATION OF TAPE SAMPLES VIA SPE

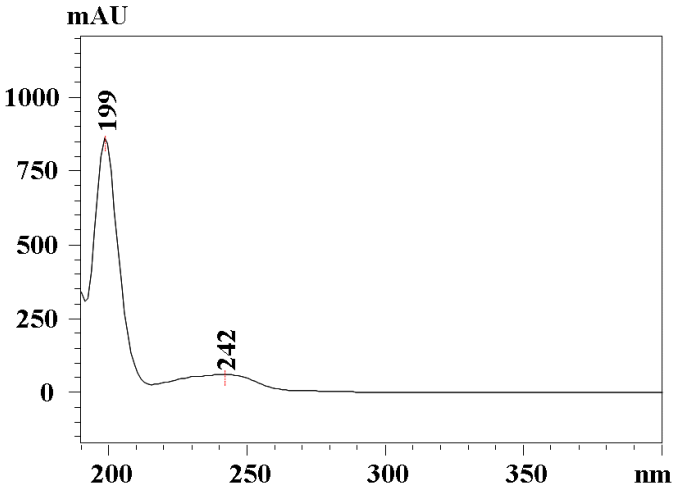
In recent decades, liquid chromatography-tandem mass spectrometry technology has become popular in impurity studies of drug substances because it can identify the molecular weight and possible molecular formula of a compound by high-resolution mass spectrometry (HRMS) and provide some structural information based on fragmentations [1-3]. However, it was rarely reported that the method was employed when the degradation of a drug occurred in a pharmaceutical preparation. One reason may be that interference with the mass spectrometry signal occurred easily as a result of complicated excipients. For instance, the polyethylene glycol chains, contained in many solvents, matrices, and surfactants showed groups of peaks during mass detection and covered the most meaningful signals (Supplementary Figure 1A). Thus, a suitable pretreatment was necessary. SPE can effectively purify and enrich the analytes. In the present study, the interference decreased and the response of drugs and impurities became clearer after the samples had been pretreated by the SPE procedure (Supplementary Figure 1B).



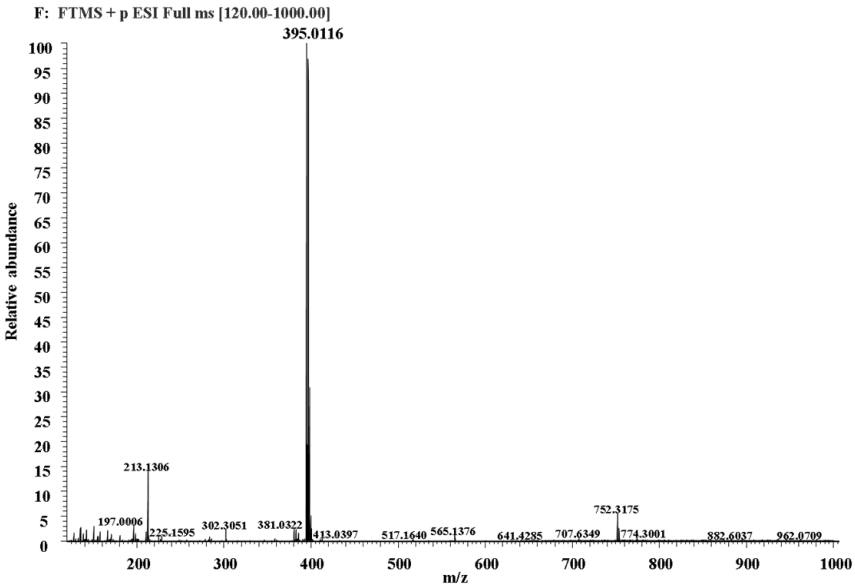
Supplementary Figure 1. Typical LC-MS chromatograms of tape samples stored at 60 °C for 20 d. (A) Pretreated by SPE; (B) Without pretreatment. LC-MS, liquid chromatography-mass spectrometry; SPE, solid-phase extraction; ECN, econazole nitrate; TA, triamcinolone acetonide; NL, normalized level; RT, retention time; TIC, total ion current; F, filter; ITMS, ion trap mass spectrometer; c ESI, capillary electrospray ionization. Peaks 1 and 2 correspond to impurity 1 and impurity 2 detected in the stability tests.

2 IDENTIFYING THE STRUCTURE OF THE SYNTHESIZED ECN-O

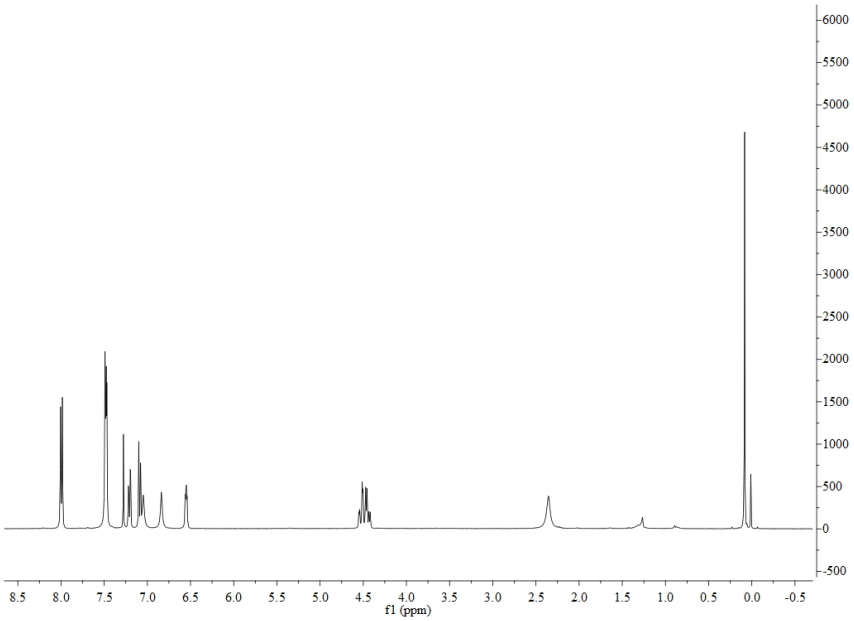
The structure of the synthesized ECN-O was identified using a photodiode array detector (PDA), HRMS, proton nuclear magnetic resonance (Supplementary Figures 2-4).



Supplementary Figure 2. UV spectrogram of synthesized ECN-O. ECN-O, the oxidative product of econazole nitrate; UV, ultraviolet.



Supplementary Figure 3. HRMS spectrogram of synthesized ECN-O. HRMS, high-resolution mass spectrometry; ECN-O, the oxidative product of econazole nitrate; FTMS, Fourier transform mass spectrometry; p ESI, positive electrospray ionization.



Supplementary Figure 4. ^{1}H NMR spectrogram of synthesized ECN-O. ECN-O, the oxidative product of econazole nitrate; ^{1}H NMR, proton nuclear magnetic resonance.

3 METHOD VALIDATION OF QUANTITATIVE ANALYSIS OF ECN-O BY HPLC-PDA

3.1 Standard solution

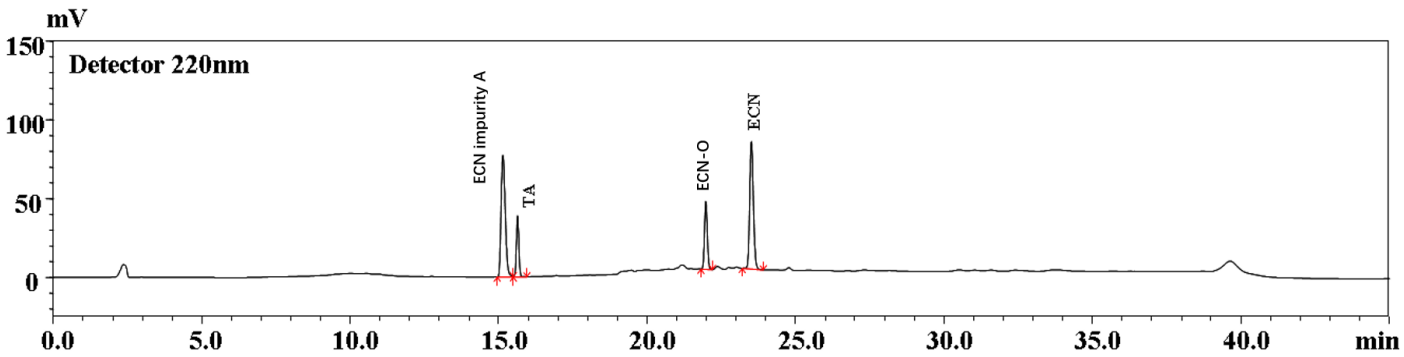
The stock solution of ECN-O was prepared in ethanol at a concentration of 500 μg/mL and serially diluted to obtain working standard solutions at concentrations of 0.2, 0.5, 1.0, 5.0, and 10.0 μg/mL.

3.2 Sample solution

Seventy micrograms of tape samples were extracted with 10 mL of ether and ethanol. The solution (0.5 mL) was mixed with water (1.0 mL) and vortexed for 60 s in a 1.5-mL Eppendorf tube to separate out the polymer excipients. Then, the mixture was filtered through a 0.45-μm microporous membrane. The final sample concentration was equivalent to 23.3 μg/mL of econazole nitrate.

3.3 System suitability

The system suitability was assessed to verify the chromatographic separation (Supplementary Figure 5). The results are shown in Supplementary Table 1.



Supplementary Figure 5. HPLC-PDA chromatogram of system suitability test for ECN, TA, ECN impurity A, and ECN-O. HPLC-PDA, high-performance liquid chromatography with photodiode array detection; ECN, econazole nitrate; TA, triamcinolone acetonide; ECN-O, the oxidative product of econazole nitrate.

Supplementary Table 1. System suitability report for ECN, TA, ECN impurity A, and the ECN-O

Analyte	Retention time (min)	Resolution	Tailing factor	Plate count
ECN impurity A	15.13	-	1.28	51,485
TA	15.64	2.30	1.02	144,922
ECN-O	21.98	36.40	1.07	227,712
ECN	23.51	7.30	1.13	159,544

Note: ECN, econazole nitrate; TA, triamcinolone acetonide, ECN-O, the oxidative product of econazole nitrate.

3.4 Linearity

The linearity was determined by the calibration curve obtained using standard solutions. The slope and other statistics of calibration curves were calculated by linear regression. The calibration curves showed good linearity in the concentration range (0.2–100.0 μg/mL), with a correlation coefficient of R^2=0.9998 (Supplementary Table 2).

Supplementary Table 2. Linearity report for ECN, TA, ECN impurity A, and ECN-O, detected at 220 nm

Analyte	Range (μg/mL)	Regression equation	Correlation coefficient	Correction factor[a]
ECN	0.7760~38.80	y=70821x+9684.40	0.9999	-
TA	0.0776~3.88	y=44073x+578.71	1.0000	0.62
ECN impurity A	0.3910~6.25	y=74704x+13747	0.9995	1.05
ECN-O	0.2000~10.00	y=56993x+2989.20	0.9999	0.80

Note: ECN, econazole nitrate; TA, triamcinolone acetonide, ECN-O, the oxidative product of econazole nitrate. [a]The correction factors are relative to ECN.

3.5 Precision

The intra-day and inter-day precisions were determined by analyzing sample solutions. For this evaluation, six sample solution replicates at 100% of the test concentration were prepared on different days. The relative standard deviation was used to report the precision. The intra-day and inter-day precisions were 1.84% and 1.43%, respectively.

3.6 Accuracy

The accuracy was evaluated by adding known amounts of standard solution to the blank tape sample solution. The accuracy was evaluated at three concentration levels (80%, 100%, and 120%) (Supplementary Table 3).

Supplementary Table 3. Accuracy report for ECN-O, n=3

Concentration (μg/mL)	Level (%)	Recovery (%)
0.4	80	101.1
0.5	100	98.9
0.6	120	99.0

Note: ECN-O, the oxidative product of econazole nitrate.

REFERENCES

[1] Jain D, Basniwal PK. Forced degradation and impurity profiling: Recent trends in analytical perspectives. J Pharm Biomed Anal. 2013 Dec;86:11-35. https://doi.org//10.1016/j.jpba.2013.07.013

[2] Dendeni M, Cimetiere N, Amrane A, Hamida NB. Impurity profiling of trandolapril under stress testing: Structure elucidation of by-products and development of degradation pathway. Int J Pharm. 2012 Nov 15;438(1):61-70. https://doi.org//10.1016/j.ijpharm.2012.08.048

[3] Kaushik D, Kaur J, Paul Kaur V, Saini B, Bansal Y, Bansal G. Forced degradation, LC–UV, MS^n and LC–MS–TOF studies on azilsartan: Identification of a known and three new degradation impurities. J Pharm Biomed Anal. 2016 Feb 20;120:202-211. https://doi.org//10.1016/j.jpba.2015.12.027