A variation of Favorskii rearrangement mechanism under weakly acidic conditions: the case of clobetasol propionate degradation in solution

Yun Tian[a,b], Haoran Zhang[a], Zach Zhu[b], Li Chen[c], Evelyn Wang[b], Jianyang Jin[c], Dan Li[c], , Wenquan Zhu[c], Min Li[c,d,*], Lei Fu[a,**]

[a] *Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China*

[b] *Prinbury Biopharm Co., Ltd., 538 Cailun Road, Pudong, Shanghai 201203, PR China*

[c] *Center of Excellence for Modern Analytical Technologies (CEMAT), Zhejiang Huahai Pharmaceutical Co. Ltd., Xunqiao, Linhai, Zhejiang 317204, PR China*

[d] *Huahai US, Inc. 700 Atrium Drive, Somerset, NJ 08873, USA*

Key words: Favorskii rearrangement; clobetasol propionate; corticosteroids; degradation pathway

Abstract

The Favorskii rearrangement is an important chemical transformation that usually occurs for α-haloketones to form carboxylic acids and their derivatives under alkaline conditions. During the stability study of a clobetasol propionate topical solution, it has been found that clobetasol propionate, a corticosteroid possessing the 20-ketone-21-chloro moiety in the 17-position of the D ring, forms a major degradant via a Favorskii-like rearrangement under weakly acidic conditions. The mechanism of this variation of the original Favorskii rearrangement is proposed and the activation energy

*Corresponding author: minli88@yahoo.com

**Corresponding author: leifu@sjtu.edu.cn

for the formation of the degradant is calculated.

Introduction

The Favorskii rearrangement is an important chemical reaction discovered in 1894[1], which describes the transformation of α-haloketones to carboxylic acids, esters and amides under the alkaline conditions such as hydroxide, alkoxide ions, ammonia and amines, respectively[2]. Two predominant mechanisms for the Favorskii rearrangement have been proposed based on various mechanistic studies[3]. One is the cyclopropanone mechanism, which involves the open 1,3-dipolar form of cyclopropanone and/or the cyclopropanone as the critical reaction intermediates when an α-hydrogen is present (Scheme 1, Pathway a). The second one is the semi-benzylic mechanism for haloketones without α-hydrogen (Scheme 1, Pathway b), which is also referred as quasi-Favorskii rearrangement[4]. Both mechanisms are operative under alkaline conditions. Various derivatives of carboxylic acids containing the same number of carbon as in the initial ketone can be obtained dependent upon the base and/or solvent used as well as the number of halogen present in the ketone[5,6].

During our recent stability studies (Table 1) of a formulated 0.05% topical solution of clobetasol propionate (**1**), a corticosteroidal drug clinically used for the treatment of dermatological diseases[7], a major degradant (**2**) was observed under acidic conditions at a level up to 1.39% by using the related substances method

(refer to Supplementary data), which exceeded the ICH identification threshold for finished dosage forms. We then identified this major degradant, which eluted at a relative retention time of 0.49 versus clobetasol propionate, using the strategy of LC-PDA/UV-MS^n analysis in conjunction with mechanism-based forced degradation and NMR spectroscopy (refer to Supplementary data)[8–17]. Upon search in the literature, it indicated that this impurity is listed as Impurity F in the European Pharmacopeia (EP). Nevertheless, this impurity has not been reported in the literature, other than its listing in the EP. Thus, it would be worthwhile to study its formation mechanism in order to control its content in our final formulation of the topic solution of clobetasol propionate. In this paper, we propose a formation mechanism of this degradation impurity from clobetasol propionate under weakly acidic conditions (Scheme 2), which is a variation of the original Favorskii rearrangement that only proceeds under alkaline conditions. The proposed mechanism is based on the evidence obtained from the mechanistic studies involving various forced degradation experiments. Understanding the root cause of degradant formation via mechanistic study would be critical in controlling impurities in drug products, thus ensuring the quality and safety of the drug products[14].

Initial forced degradation study

Forced degradation on clobetasol propionate was conducted (Table 2) to investigate the formation mechanism of the degradant at RRT 0.49. Approximately 5 mg of clobetasol propionate was dissolved in a mixture of alcohol/H_2O (40/60, v/v) and subject to weak acid (acetic acid), strong acid (HCl) and alkaline (NaOH) conditions, respectively. The results show that RRT 0.49 impurity was the major degradation product formed (1.62%), when clobetasol propionate was dissolved in the isopropanol/water mixture in the presence of acetic acid at 60°C for 14 days (**Fig. 2**). On the contrary, under either the alkaline condition (10 mM NaOH, 3 mL) at room temperature for 2 h or strong acidic condition (1 M HCl, 3mL) at 80℃ for 4 h, appreciable decomposition of clobetasol propionate was observed in both cases: the total degradants formed were 6.3% in the alkaline solution and 5.4% in the acidic solution, respectively. Nevertheless, no RRT 0.49 impurity was detected in either of the forced degradation solutions.

Based on the above forced degradation experiments, a solution containing clobetasol propionate (5.0 g), isopropanol (200 mL), water (50 mL) and acetic acid (0.33 mL) was heated at 80℃ for 10 days, during which time RRT 0.49 impurity grew to 2.7%. The impurity was isolated from a portion of the forced degradation sample solution by semi-preparative HPLC (20 mg, HPLC purity ~96%) and subject to 1D and 2D NMR structure elucidation. The structure of RRT 0.49 impurity was identified

to be (11β,16β)-9-fluoro-11-hydroxy-16-methyl-3-oxopregna-1,4,17-triene-21-oic acid (**2**, **Fig. 1**) based on the LC-PDA/UV-MS^n and NMR results (refer to Supplementary data). Upon search in the literature, it indicated that the formation mechanism of this impurity has not been reported. Thus, it would be worthwhile to study its formation mechanism in order to control its content in our final formulation of the topic solution of clobetasol propionate.

The formation of RRT 0.49 impurity seems to correlate with the use of acetic acid as it provides a weakly acidic condition (the pKa of acetic acid is 4.8)[18]. When acetic acid was replaced by phosphate buffer and the pH of the resulting solution was adjusted to ~4.6 with phosphoric acid, a similar amount of RRT 0.49 impurity was formed in the clobetasol solution, indicating a weakly acidic condition can facilitate the formation of RRT 0.49 impurity. In addition, the same degradation reaction occurred when isopropanol was replaced by methanol, while the pH remained the same. Hence, it is clear that RRT 0.49 impurity is formed when a solution of clobetasol propionate in a mixture of alcohol (isopropanol or methanol) and water is subject to a weakly acidic condition under elevated temperature.

Proposed formation mechanism of RRT 0.49 impurity via Favorskii intermediate

Based on the above studies, a plausible degradation mechanism can be

proposed as follows: under the weakly acidic condition, the 20-ketone moiety of clobetasol propionate undergoes enolization. The enol thus formed is nucleophilic and is capable of attacking the 17-position, which would result in the elimination of the 17-propionyl group and concurrent formation of the Favorskii intermediate of 20-oxo-21-chloro-cyclopropanone ring (Scheme 2). The Favorskii intermediate can then be attacked by water, followed by rearrangement and elimination of HCl to yield RRT 0.49 impurity. In this process, the formation of RRT 0.49 impurity would require a weakly acidic condition to facilitate enolization as well as the subsequent protonation and eventual elimination of the 17-propionyl group, both of which steps are critical for the key Favorskii intermediate to form.

Second stage of forced degradation for mechanism study

Based on the above proposed mechanism, it would be difficult for clobetasol, the parent compound of clobetasol propionate, to undergo the same transformation to produce RRT 0.49 impurity, because clobetasol lacks a good leaving group such as the propionyl group at the 17-position. To test this projection based on the proposed mechanism, clobetasol (**3**) was subject to the same weakly acidic forced degradation condition; indeed, no RRT 0.49 impurity was formed, which is consistent with the projection. It appears that the propionyl group would be a better leaving group than the hydroxyl group within the steroidal core under the weakly

acidic condition.

To further study if RRT 0.49 impurity may be formed as a degradant in other corticosteroids that contain an electrophilic group at the 17-position and potential leaving group at the 21-position, betamethasone dipropionate and mometasone furoate were selected and subject to the same forced degradation condition (Table 2). RRT 0.49 impurity was not formed in either of the stressed solutions of betamethasone dipropionate (**4**) and momentasone furoate (**5**). Betamethasone dipropionate[19,20] is structurally identical to clobetasol propionate, except for the substituent at the 21-position. Hence, with betamethasone dipropionate, its enol form may be less nucleophilic, due to the electron-withdrawing effect of the 21-propionyl group, for it to launch the nucleophilic attack to form the critical Favorskii intermediate. With mometasone furoate[21], its 17-furoyl group may be more difficult to eliminate than the 17-propionyl group (in clobetasol propionate), which may be due to the fact that the ester of an aromatic acid is usually more stable than its counterpart of an aliphatic acid (hence, the linkage between the steroidal core and the 17-furoyl group would be stronger in the case of mometasone furoate). These results are consistent with the proposed mechanism in which the co-presence of the 21-chloro-20-ketone and 17-propionyl (an aliphatic acyl) moieties is critical for the formation of RRT 0.49 impurity through the Favorskii-like rearrangement mechanism (Scheme 2).

Kinetic study of RRT 0.49 impurity formation

The growth trend of RRT 0.49 impurity in the finished dosage form of 0.05% solution is found to be linear under the ICH long term, intermediate, and accelerated stability conditions (Table 1), respectively, which indicates that the formation of RRT 0.49 impurity is a nominal zero order reaction during the ICH stability conditions. Hence, the Arrhenius equation[22–24] was used to study the kinetic behavior of RRT 0.49 impurity formation in the clobetasol propionate topical solution.

$$k = Ae^{-Ea/RT} \qquad (1)$$

$$\text{or} \quad \ln k = \frac{-Ea}{R}\frac{1}{T} + \ln A \qquad (2)$$

Where:

k is the rate constant,

T is the absolute temperature (in Kelvin),

A is the pre-exponential or frequency factor, a constant for each chemical reaction. According to the collision theory, A is the frequency of collisions in the correct orientation,

E_a is the activation energy for the reaction,

R is the universal gas constant.

The E_a (activation energy) can be obtained based on the linear plot by using the Arrhenius equation (2). Hence, E_a is calculated to be 116.9 kJ·mol^{-1} (or 28.0 kcal·mol^{-1}). The kinetics plot and Arrhenius plot are shown in Supplementary data, Fig. S4.

Conclusions

A major degradant of clobetasol propionate was observed at RRT 0.49 during the stability studies of a clobetasol propionate topical solution (0.05%, w/v). Through a comprehensive investigation, a novel mechanism for the formation of RRT 0.49 impurity, as the major degradant of clobetasol propionate under the weakly acidic conditions, is proposed in which the key Favorskii intermediate plays a critical role. This variation of the original Favorskii rearrangement has not been reported before. The finding of the novel degradation pathway via the variation of the original Favorskii rearrangement mechanism should enrich the degradation chemistry of corticosteroids containing α-haloketone moieties in their 17-position and thus help ensure the quality and safety of these drug products.

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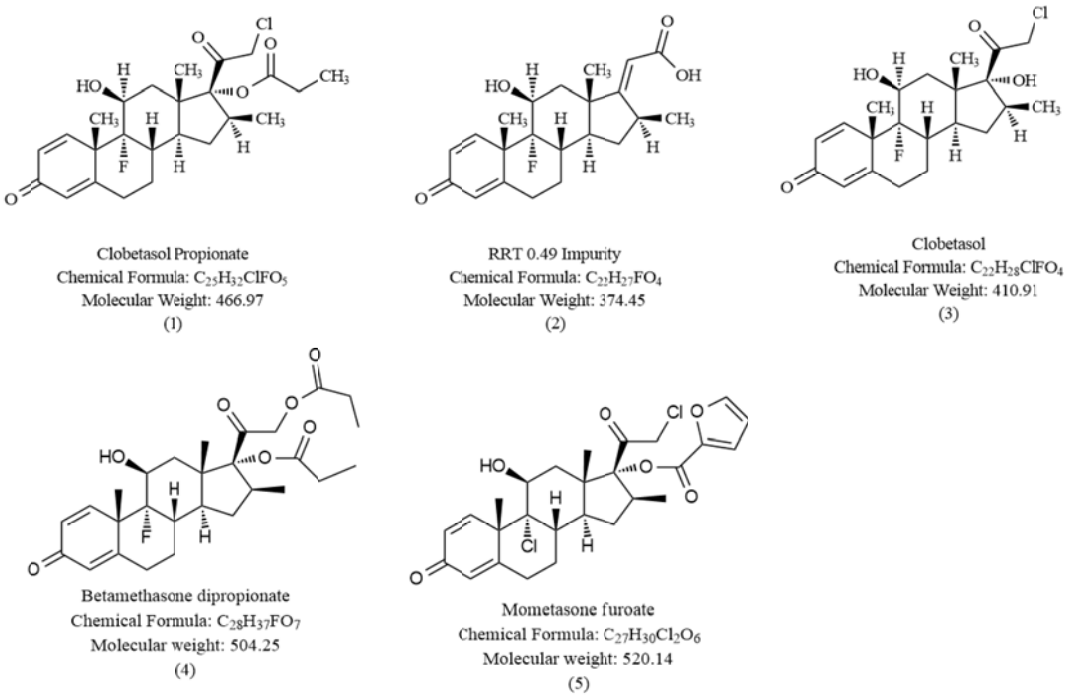


Figure 1. Structures of the compounds related to this study: (1) Clobetasol propionate. (2) RRT 0.49 impurity (EP impurity F). (3) Clobetasol. (4) Betamethasone dipropionate. (5) Mometasone furoate.

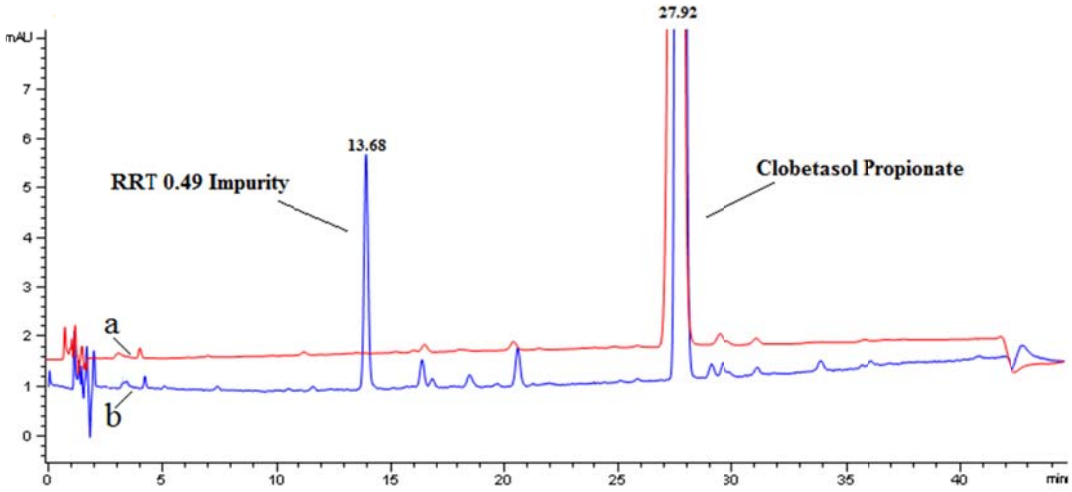


Figure 2. HPLC chromatograms of the 0.05% (w/v) clobetasol propionate solution in isopropanol/H_2O/Acetic acid (60/40/0.13, v/v/v) before (a) and after (b) the forced degradation at 60°C for 14 days. The RRT 0.49 unknown peak (1.62%) eluted at 13.68 min, while clobetasol propionate eluted at 27.92 min. The solutions were analyzed by the related substances method with the mobile phase B (CH_3CN) varied according to the following gradient program: 0 min (30% B), 3 min (30% B), 40 min (60% B), 40.1 min (30% B), and 45 min (30% B). The mobile phase A was 0.05 M NaH_2PO_4 (pH 2.5)/MeOH (4/1, v/v). The method was run at a flow rate of 1.0 mL/min with the detection wavelength at 240 nm. The sample was prepared in diluent (mobile phase A/B, 1/1, v/v) at a concentration of 0.1 mg/mL.

Table 1. Peak area% of RRT 0.49 Impurity in stability samples of the 0.05% clobetasol propionate topical solution under different stability conditions

Temperature (℃)	Humidity (%)	Duration	RRT 0.49 Impurity
40	75	Initial	ND
		0.5 month	0.26%
		1 month	0.50%
		2 months	0.97%
		3 months	1.39%
30	65	Initial	ND
		0.5 month	0.08%
		2 months	0.26%
		3 months	0.33%
25	60	Initial	ND
		0.5 month	0.05%
		1 month	0.07%
		3 months	0.16%
		6 months	0.30%

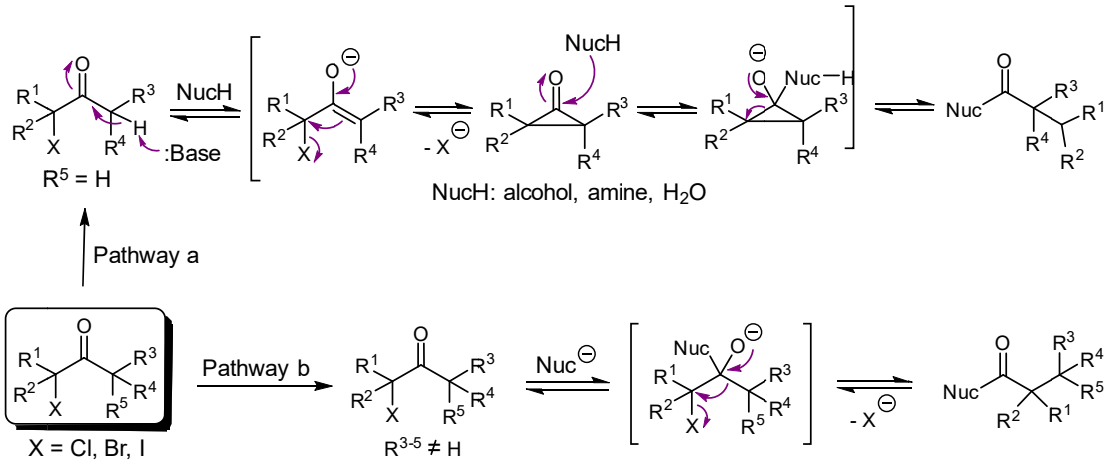
Notes: ND, not detected. The clobetasol propionate topical solutions (0.05%, w/v) were placed under the ICH accelerated, intermediate and long-term stability conditions, and pulled for analysis at certain time points. The stability samples were analyzed by the HPLC related substances method. The growth trends of RRT 0.49 impurity were found to be linear under all the three stability conditions.

Table 2. Forced degradation for mechanistic study

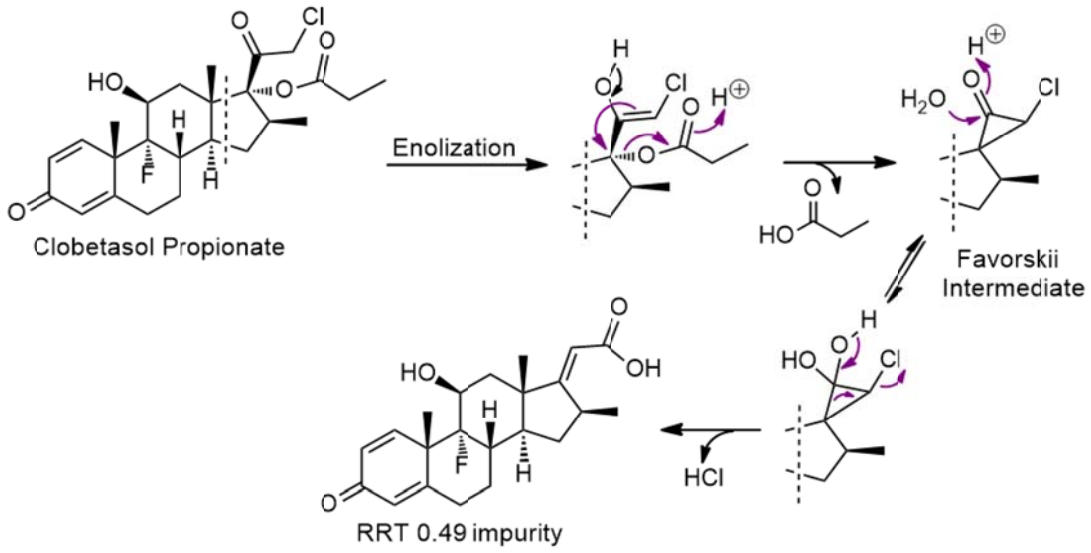
Compound	Functional groups			Solvent/condition*	RRT 0.49
Clobetasol propionate	17-propionate	20-ketone	21-Cl	Composition A, 60℃ for 14 days	√
				Composition B,60℃ for 14 days	√
				Composition C, 60℃ for 14 days	√
				MeOH, 3mL 1N HCl, at 80℃ for 4 hr	×
				MeOH, 3mL 0.01 N NaOH, at RT for 2 hr	×
				MeOH, UV light, 2hr	×
Clobetasol	17-hydroxyl	20-ketone	21-Cl	Composition A, 60℃ for 14 days	×
Betamethasone dipropionate	17-propionate	20-ketone	21-propionate	Composition A, 60℃ for 14 days	×
				Composition D, 60℃ for 14 days	×
Mometasone furoate	17-furoate	20-ketone-	21-Cl	Composition A, 60℃ for 14 days	×

Notes: Composition A is IPA-H_2O-Acetic acid (40/60/0.13 v/v/v); Composition B is MeOH-H_2O-Acetic acid (40/60/0.13 v/v/v); Composition C is IPA-H_2O-NaH2PO4/Phosphate acid (40/60/0.13 v/v/v); Composition D is IPA-H_2O-Acetic acid/NH4Cl (40/60/0.13/ v/v/v/v). Abbreviation: IPA, isopropanol; MeOH, methanol; RT, room temperature. Sample preparations: 5 mg of each compound was added into 20-mL amber glass vials, respectively, followed by addition of the above Composition A/B/C/D, respectively, into each amber glass vial and the resulting mixtures were mixed well. The samples were placed at 60℃ for 14 days and analyzed by the related substances method.

√ means RRT 0.49 impurity was formed in the sample solution; × means it was not formed



Scheme 1. The two predominant mechanisms of the Favorskii rearrangement[3] for α-haloketones with α-hydrogen (Pathway a) and without α-hydrogen (Pathway b, which is also referred to as quasi-Favorskii rearrangement).



Scheme 2. Proposed formation mechanism of RRT 0.49 impurity, the major degradant in 0.05% clobetasol propionate topic solution, under weakly acidic conditions.

Supplementary data

List of supplementary data:

*Corresponding author: minli88@yahoo.com

**Corresponding author: leifu@sjtu.edu.cn

By applying LC-PDA/UV-MS^n analysis in conjunction with mechanism-based forced degradation and NMR spectroscopy, RRT 0.49 impurity was identified to be (11β,16β)-9-fluoro-11-hydroxy-16-methyl-3-oxopregna-1,4,17-triene-21-oic acid, the experimental part and the results are described in Sections 1 to 6 below.

Experimental

1. Related substances method

The forced degradation samples were analyzed by the related substances method. The related substances method was performed on an Agilent 1260 high performance liquid chromatography instrument equipped with a PDA detector operated in both scan mode and a fixed wavelength of detection at 240 nm. The HPLC column was Agilent ZORBAX SB-C18, 150×4.6 mm, 5 μm column and column temperature was set at 35°C. The mobile phase consisted of A (0.05 M NaH_2PO_4 buffer/methanol, 4/1, v/v) and B (acetonitrile), and the analysis was effected by a gradient with the percentage of the mobile phase B varied according to the following program: 0 min (30% B), 3 min (30% B), 40 min (60% B), 40.1 min (30% B), and 45 min (30% B). The method was run at a flow rate of 1.0 mL/min. The sample was prepared in diluent (mobile phase A/B, 1/1, v/v) at a concentration of 0.1 mg/mL.

2. LC-PDA/UV-MS^n analysis

To identify the RRT 0.49 unknown impurity, an Agilent HPLC instrument (1260 series, Agilent Technologies, USA) interfaced to a quadrupole time-of-flight (Q-TOF) mass spectrometer (6545 series, Agilent Technologies, USA) was used for the LC-PDA/UV-MS^n analysis of the stability samples. The chromatographic conditions of the

LC-PDA/UV-MS^n method were developed based on the HPLC method for related substances determination, except that the HPLC column was replaced by a Waters Xterra RP18, 150 x 4.6 mm, 3.5 µm column and the mobile phase A was replaced by 0.1% formic acid in water. The Q-TOF mass spectrometer was operated at positive ESI mode with the following parameters: nebulizer pressure 60 psi, sheath gas temperature 350°C, sheath gas flow 12 L/min, fragmentor voltage 90 V, and capillary voltage +3000 V. The acquisition range was 100-1000 Da and for the MS^2 analyses, the collision energy was set at 10, 20, and 30 eV, respectively.

3. Preparation and Purification of RRT 0.49 impurity from Forced Degradation

To prepare a suitable amount of pure RRT 0.49 impurity for NMR structural elucidation, a forced degradation of clobetasol propionate was designed to enrich and isolate RRT 0.49 impurity. Hence, a solution containing clobetasol propionate (5.0 g), isopropanol (200 mL), water (50 mL) and acetic acid (0.33 mL) was heated at 80°C for 10 days, during which period RRT 0.49 impurity grew to 2.7%. RRT 0.49 impurity was then isolated from a portion of the forced degradation sample solution by semi-preparative HPLC, which was performed on a Shimadzu LC-8A system (Model XXI-4563) with a Waters Xterra RP18, 150 mm × 19 mm, 5 µm semi-preparative HPLC column. The separation was carried out at room temperature, and the same mobile phase system as used in LC-PDA/UV-MS^n analyses was utilized as per the gradient according to the following program: 0 min (40% B), 30 min (40% B), 35 min (55% B), 35.1 min (80% B), and 41 min (80% B). The HPLC separation was run at a flow rate of 9 mL/min with an injection volume of 1.8 mL and the detection wavelength for fraction collection

was set at 240 nm. Approximately 20 mg of purified RRT 0.49 impurity was obtained with an HPLC purity of ~96%.

4. 1D and 2D NMR Measurement

Approximately 20 mg of the above isolated RRT 0.49 impurity was dissolved in 1 mL DMSO-$_{d6}$. ^{1}H, ^{13}C NMR and 2D NMR spectra of RRT 0.49 impurity were acquired on an Agilent 400 MHz NMR spectrometer at 25°C. ^{1}H and ^{13}C resonances were assigned by using 2D NMR experiments: gCOSY, gHSQC, and gHMBC.

Results and discussion

5. LC-PDA/UV-MS^{n} results

The major degradant at RRT 0.49 was originally observed at 13.68 min during the related substances analysis of the accelerated stability samples of clobetasol propionate topical solution (0.05%, w/v) and its relative retention time (RRT) versus that of clobetasol propionate (27.92 min) was 0.49. The UV spectrum of clobetasol propionate showed a maximum absorbance band at 240 nm, which is typical for this family of corticosteroids due to the cross-conjugated system in the A-ring of the steroid. On the other hand, the UV spectrum of RRT 0.49 impurity showed a maximum absorbance band at 232 nm, indicating that the two compounds have different chromophores or a new chromophore may be formed in RRT 0.49 impurity **(Fig. S1)**.

RRT 0.49 impurity displayed accurate m/z values at 375.1975 and 397.1792, corresponding to its protonated and sodiated ions, respectively. The m/z value of the protonated RRT 0.49 impurity, 375.1975, matched a formula of $C_{22}H_{27}FO_4$ with an error

of 2.4 ppm. The MS^2 spectra of the protonated 0.49 impurity (m/z 375) displayed the characteristic neutral loss of HF (m/z 375 → m/z 355) and a few fragment ions that were also present in the MS^2 spectrum of clobetasol propionate, such as m/z 355, m/z 337, m/z 319, and m/z 147 under the collision energy of 10 eV (**Fig. S2**), suggesting that RRT 0.49 impurity may have similar core structure as clobetasol propionate.

6. Structure Characterization by 1D and 2D NMR Spectroscopy

After isolation by semi-preparative HPLC, ~20 mg of purified RRT 0.49 impurity was made available for analysis by 1D and 2D NMR spectroscopy. All the ^{1}H NMR and ^{13}C NMR data of clobetasol propionate and RRT 0.49 impurity are summarized in **Table S1.**

All the 22 carbons of RRT 0.49 impurity were well resolved in the ^{13}C NMR spectrum. They can be classified by their chemical shifts and gHSQC spectra into the following categories: three methyl groups (δ_C 19.7, 21.3 and 22.9), four methylene groups (δ_C 27.0, 30.1, 32.9 and 40.5), eight methine groups (δ_C 33.2, 35.9, 45.9, 70.4, 109.6, 124.2, 129.1 and 152.6) including one hydroxy methine group (δ_C 70.4) and four aromatic methine groups (δ_C 109.6, 124.2, 129.1 and 152.6), and seven quaternary carbons (δ_C 45.3, 47.9, 101.6, 166.8, 167.4, 177.5 and 185.2) including two high field quaternary carbons (δ_C 45.3 and 47.9) and one carbonyl carbon atom (δ_C 185.2).

The ^{1}H NMR spectrum displayed the characteristic signals of three methyl protons (δ_H 1.10, 1.49 and 1.23) and two sets of protons of A ring (δ_H 6.00, 6.21 and 7.29),

indicating that the impurity bears some structural similarity to that of clobetasol propionate. The main difference, nevertheless, exists in their D rings. Specifically, the gHMBC spectrum of the impurity (**Fig. S3**) revealed the correlations from H-20 (δ_H 5.33) to C-16 (δ_C 35.9), C-13 (δ_C 45.3), C-21 (δ_C 167.4), and C-17 (δ_C 177.5), respectively, which is consistent with the impurity structure that contains a conjugated enoic acid.

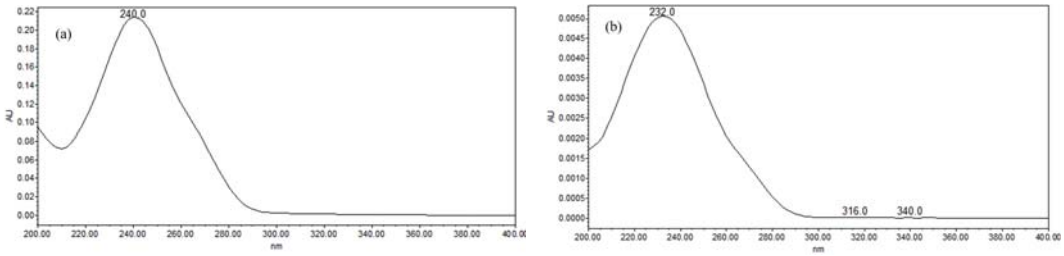


Fig. S1. UV Spectra of clobetasol propionate (a) and RRT 0.49 impurity (b).

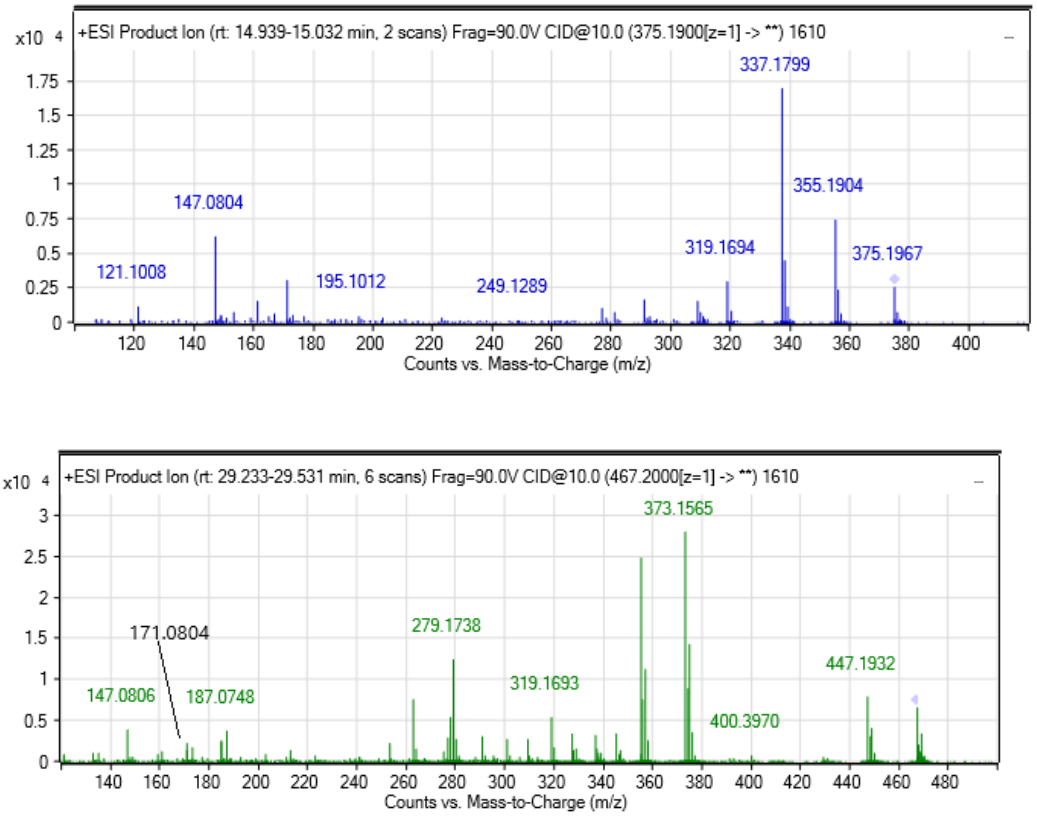
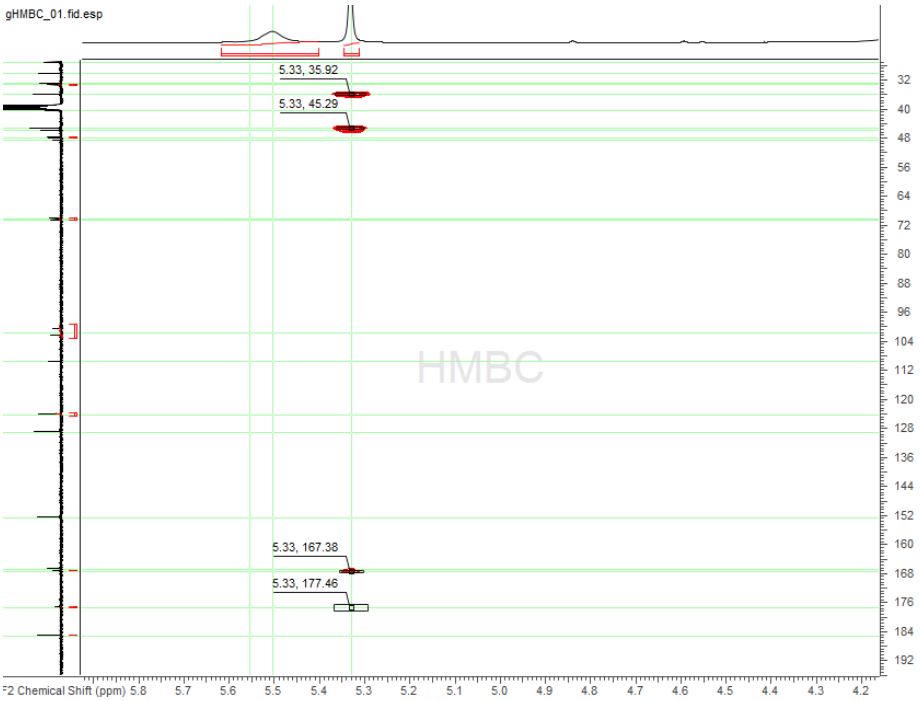


Fig. S2. MS^2 spectra of RRT 0.49 impurity (upper; m/z 375 →) and clobetasol propionate (lower; m/z 467 →) under a collision energy of 10 eV.



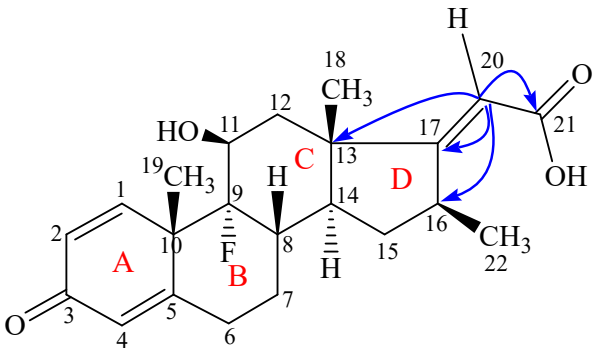
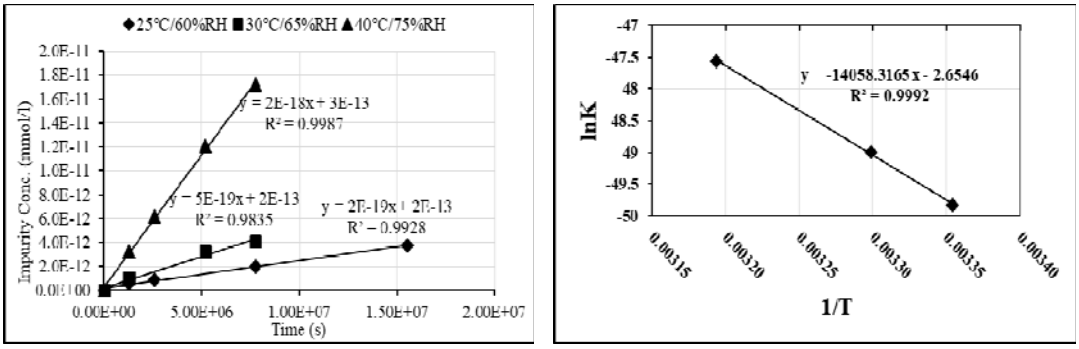


Fig. S3. gHMBC spectrum of RRT 0.49 impurity.

7. Kinetic study



(a) (b)

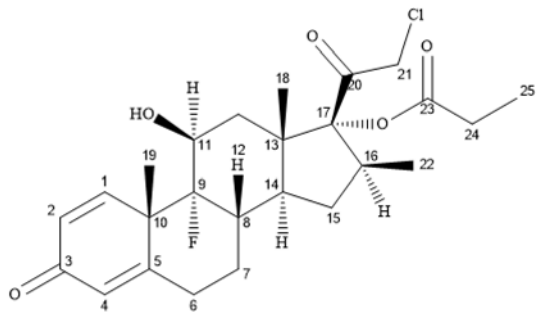
Fig. S4. (a) The kinetic plots of RRT 0.49 impurity over the courses of long term, intermediate and accelerated stability conditions, respectively. (b) The Arrhenius plot for the formation of RRT 0.49 impurity based on the kinetic plots of the stability test results.

Table S1. ^{1}H and ^{13}C NMR data of Clobetasol Propionate and RRT 0.49 impurity in DMSO-d_6 (in ppm).

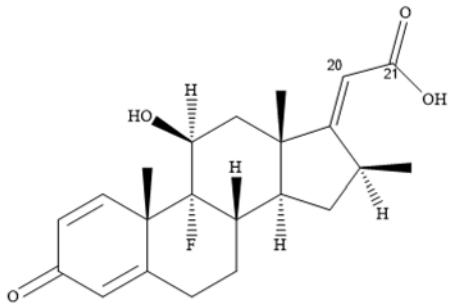
Position	Clobetasol Propionate			Impurity at RRT 0.49[1]		
	H shifts	C shifts	*J* (C, F) (Hz)	H shifts	C shifts	*J* (C, F) (Hz)
1	7.28 d (9.8)	153.0		7.29 d (10.2)	152.6	
2	6.23 d (10.0)	130.0		6.21 d (10.2)	129.1	
3	N.A.	185.7		N.A.	185.2	
4	6.02 s	124.6		6.00 s	124.2	
5	N.A.	167.2		N.A.	66.8	
6a	2.34 m	30.6		2.32 dd (13.5, 3.3)	30.1	
6b	2.63 td (13.2,5.7)			2.63 td (13.2, 5.7)		
7a	1.38 m	28.0		1.34 m	27.0	
7b	1.86 m			1.86 m		
8	2.50 m	33.4	19.1	2.47 m	33.2	19.8
9	N.A.	101.4	174.7	N.A.	101.6	175.5
10	N.A.	48.2	22.7	N.A.	47.9	22.0
11	4.20 m	70.6	35.9	4.14, m	70.4	36.5
12a	1.56 d (13.3)	36.7		1.42 d (13.3)	40.5	
12b	2.23 d (13.7)			1.86 d (12.8)		
13	N.A.	47.4		N.A.	45.3	
14	1.86 m	43.6		1.26 m	5.9	
15a	1.12 m	34.9		1.18 m	32.9	
15b	1.87 m			1.93 m		
16	2.05 m	46.8		3.12 m	35.9	
17	N.A.	94.2		N.A.	177.5	
18	0.85 s	17.4		1.10 s	19.7	

19	1.48 s	23.3	5.3	1.49 s	22.9	5.4
20	N.A.	197.9		5.33 s	109.6	
21	4.20 s	48.2		N.A.	167.4	
22	1.26 d (7.4)	20.2		1.23 d (7.0)	21.3	
23	N.A.	175.1				
24	2.39 q (7.4)	27.7				
25	1.01 t (7.4)	9.0				
11-OH	5.44 d (3.13)			5.50 br s		

Note: The numbering of the carbon skeletons of clobetasol propionate and RRT 0.49 impurity is shown as follows:



Clobetasol Propionate



RRT 0.49 Impurity