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A prediction system of oxidation reaction as a solid-state stress condition: Applied to a pyrrole-containing pharmaceutical compound

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ABSTRACT

Stress conditions for predicting oxidative degradation products in solid-state pharmaceutical compounds were investigated. 4-Methyl-2-(3,4-dimethylphenyl)-1-(4-sulfamoylphenyl)pyrrole, Compound A, was used as the model compound for this study and its four main degradation products were due to oxidation, as identified by LC–MS and LC-^{1}H NMR. In order to develop a prediction system for the oxidation reaction, solid-state Compound A was stored under moisture-saturated conditions. Hydrogen peroxide was added to the solution used to saturate the headspace with moisture and oxygen was substituted for the headspace air, in order to stimulate the oxidation reaction. After optimizing the conditions, a similar degradation product profile to that actually observed in the stability studies was obtained in only 3 days under conditions using 3% hydrogen peroxide at 40 °C. The prediction of the oxidative degradation products in a solid-state pharmaceutical compound was successfully achieved in a short term utilizing this newly developed prediction system.

1. Introduction

In order to ensure the safety of pharmaceuticals throughout their shelf life, it is necessary to adequately control the degradation products generated during the entire storage period. Thus, the conditions for the stability tests for drug substances and drug products that are sufficient for registration applications are defined in ICH Guideline Q1A (R2) [1], and the method for establishing the shelf life so as to properly ensure product quality is also indicated in ICH Guideline Q1E [2]. The thresholds for identifying and qualifying impurities, including degradation products, are also specified by ICH Guidelines Q3A (R2) and Q3B (R2) [3,4].

In the early stage of drug development, it is important to obtain information on stability and degradation mechanisms so as to develop stable pharmaceutical formulations and establish suitable packaging configurations based on this information. However, in order to elucidate the profile of degradation products generated during the storage period, it is necessary to carry out long-term stability studies under ICH storage conditions. Therefore, the development of suitable stress conditions to predict the possible degradation products in the short term is necessary.

Forced degradation studies are generally carried out to predict potential degradation products in the short term and, while various conditions are used, many such tests are performed in a solution system [5]. However, the differences in the degradation mechanisms between a solution state and a solid state often result in differing degradation product profiles. Thus, in the case of a pharmaceutical being developed in solid form, it is desirable to be able to obtain information on the stability in a solid state. In a case where forced degradation studies are performed in a solid state, conditions of high humidity or high temperature are generally applied [6–9]. However, a degradation product profile obtained under overly severe conditions may differ from the one which would be obtained under actual storage conditions due to secondary degradation. Accordingly, it is necessary to establish adequate stress conditions to predict the degradation products observed during the actual storage of pharmaceuticals in the short term.

Since oxidation, hydrolysis, dehydration and photolysis are known as the main degradation mechanisms of pharmaceuticals [10], it is important to select stress conditions that are appropriate for the degradation mechanism to be predicted. For the evaluation of hydrolysis, it is possible to verify hydrolytic reactivity by investigating stability in solutions or suspensions in a wide pH range. For

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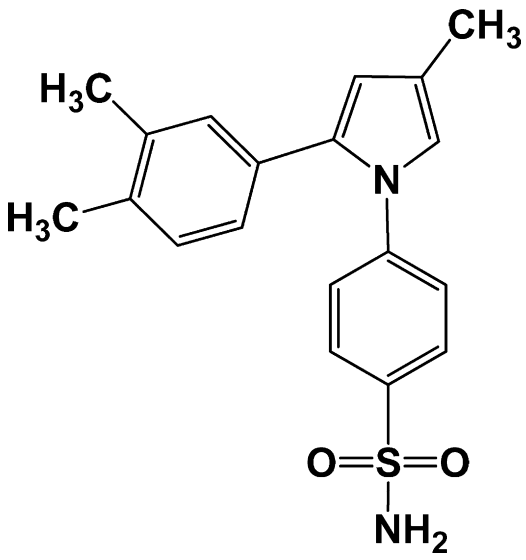

Fig. 1. Chemical structure of Compound A.

the evaluation of dehydration, thermal stress conditions are often used, and for photolysis, it is possible to evaluate photostability characteristics by exposing the pharmaceutical to an appropriate light source at a suitable illumination intensity. Oxidative degradation is one of the most frequently occurring mechanisms of pharmaceutical degradation and stability and methods for stability evaluation have been widely researched [11,12]. In research conducted in a solution state, systems featuring the addition of the oxidant hydrogen peroxide, the radical initiator azobisisobutylonitrile (AIBN), transition metals such as iron and copper, which accelerate oxidation by the transfer of electrons, or rose bengal, a photosensitizer known for generating singlet oxygen, have been reported [13–15], and evaluation systems based on the oxidation mechanisms are being standardized [5,16]. Nevertheless, since degradation mechanisms often differ between a solution state and a solid state, in some cases it is difficult to predict the most likely degradation products that will be generated in solid pharmaceuticals under actual storage conditions based upon the forced degradation studies in a solution state. It has also been reported that the mechanism of the oxidation reaction will differ depending upon the type of solvent used [17,18]. In research conducted in a solid state, on the other hand, studies investigating the effect of oxygen in the headspace of the storage system have been only reported for oxidative stability evaluation [5,19].

Therefore, in this study we investigated stress conditions to predict oxidative degradation products in the short term using a model compound, 4-methyl-2-(3,4-dimethylphenyl)-1-(4-sulfamoylphenyl)pyrrole, namely Compound A, previously under development as a selective cyclooxygenase-2 (COX-2) inhibitor.

As shown in Fig. 1, Compound A contains a pyrrole ring in its structure and has extremely low solubility in water (0.05 μg/mL). Prediction of the degradation product profile in the short term was attempted by storing solid-state Compound A under moisture-saturated conditions. Prediction systems using aqueous solutions to which were added either the oxidant hydrogen peroxide or rose bengal, a photosensitizer known to generate singlet oxygen upon irradiation with light, were also investigated.

2. Experimental

2.1. Chemicals and reagents

4-Methyl-2-(3,4-dimethylphenyl)-1-(4-sulfamoylphenyl)pyrrole, Compound A, was synthesized by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). The Compound A tablets were also produced by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Guaranteed Reagent hydrogen peroxide (30%, v/v) and rose bengal were purchased from Wako Pure Chemicals, Ltd. (Osaka, Japan). Formic acid for LC–MS use was also purchased from Wako Pure Chemicals, Ltd. (Osaka, Japan). Deuterium oxide (D_2O) and acetonitrile-d_3 (CD_3CN) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). The water was purified with a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA).

2.2. Stability studies

The Compound A drug substance was stored for 16 weeks under separate conditions of 60 °C and 40 °C/75% RH. The Compound A tablets were kept for 4 weeks under separate conditions of 60 °C and 40 °C/75% RH.

2.3. Forced degradation studies under moisture-saturated conditions

Compound A in the amount of 15 mg was weighed into 20-mL amber glass bottles. Water and hydrogen peroxide solution of 10 mL were separately poured into 50-mL glass centrifuge tubes. The 20-mL amber glass bottles containing Compound A were placed, uncovered, into 50-mL glass centrifuge tubes to which these aqueous solutions had been added, so that the aqueous solutions surrounded the 20-mL amber glass bottles. The lids of the 50-mL glass centrifuge tubes were closed, the space inside the tubes was saturated with moisture, and solid-state Compound A was stored therein. In order to investigate the effects of oxygen, samples in which oxygen was substituted for the ambient air in the headspace of the 50-mL glass centrifuge tubes were also prepared. To optimize the conditions, hydrogen peroxide concentrations of 0.3%, 3%, and 30% (v/v) were employed, along with storage temperatures of 25 °C, 40 °C, and 60 °C. The lifetime of singlet oxygen is known to be longer in deuterium oxide than in water [20]. For this reason, the photosensitizer rose bengal, which is known to generate singlet oxygen upon irradiation with light, was dissolved in deuterium oxide. A sample was prepared according to the same method described above using 5 mg of Compound A, which was stored under moisture-saturated conditions of rose bengal solution. Compound A was stored at 40 °C with various concentrations of rose bengal containing 0.1 mmol/L, 1 mmol/L, 10 mmol/L and 100 mmol/L. During storage, the rose bengal solution was illuminated with a fluorescent lamp at 2500 lux.

2.4. Preparation of sample solution

Following storage, the sample was dissolved in a mixture of water–acetonitrile (1:1, v/v), and the concentration of Compound A was adjusted to 1.2–1.5 mg/mL.

2.5. HPLC-UV analysis

The samples were separated on an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of an online degasser G1379A, a binary pump G1312A, an autosampler G1329A, a column compartment G1316A and a diode array detector G1315B. Reversed-phase chromatographic separation was achieved using an Agilent ZORBAX SB-C8 column (1.8 μm in particle size, 2.1 mm in inside diameter and 10 cm in length) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The column temperature was kept constant at 70 °C. The mobile phase composition was linearly ramped from 45% to 50% of mobile phase B over the 20 min after injection with a flow rate of 0.3 mL/min. Two-microliter aliquots of each sample solution were

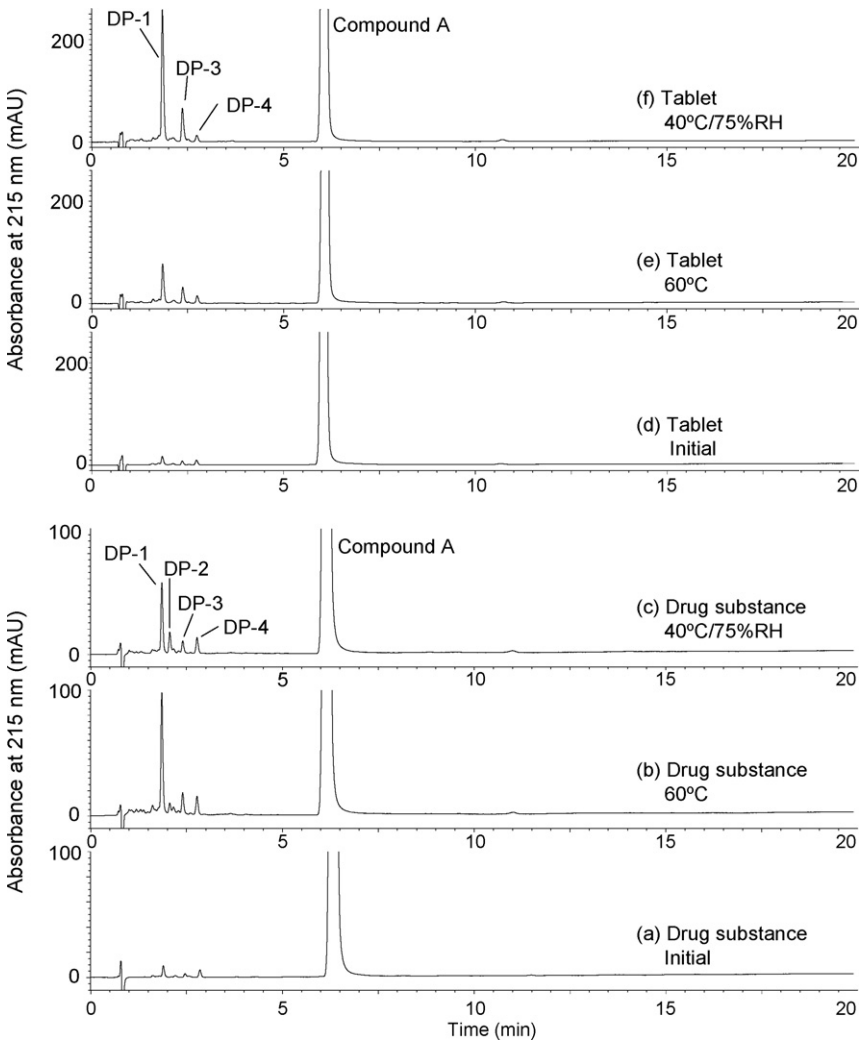

Fig. 2. Chromatograms of Compound A drug substance stored at 60 °C and 40 °C/75% RH for 16 weeks and its tablets stored at 60 °C and 40 °C/75% RH for 4 weeks.

injected into the system and the detection was carried out at UV 215 nm.

2.6. Identification of degradation products

2.6.1. LC–MS analysis

The liquid chromatograph used was the Agilent 1100 HPLC system described previously. The HPLC separation was conducted under the same conditions as those used for the LC-UV analysis. A 2 μL aliquot of the sample solution was subjected to analysis.

A hybrid quadrupole time-of-flight mass spectrometer Q-TOF Premier with Lockspray™ (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source was used and operated in positive ESI mode. Nitrogen generated from pressurized air in a nitrogen generator from Nitrogen Generator System (Whatman Inc., Haverhill, MA, USA) was used with flow rates of 50 L/h for cone gas and 400 L/h for desolvation gas. The capillary voltage was 3.0 kV and the cone voltage was 15 V. The source and desolvation temperatures were 120 °C and 200 °C, respectively. For the MS/MS operation, argon was used as a collision gas.

2.6.2. LC-^{1}H NMR analysis

The LC-NMR experiments were performed in stopped-flow mode on an Avance 600 spectrometer (Bruker BioSpin, Rheinstetten, Germany) working at 600.13 MHz for ^{1}H, coupled to the Agilent 1100 HPLC system described in Section 2.5 and a Bruker peak sampling unit (BPSU-12) interface. The HPLC separation was conducted under the same conditions as those used for the LC-UV analysis, except for the use of deuterated solvents of D_2O and CD_3CN in the

Table 1
Result of accurate mass measurement for Compound A and its degradation products.

Compound	Observed mass	Calculated mass	Error (ppm)	Molecular formula $[M+H]^+$	Difference[a]
Compound A	341.1318	341.1324	−1.8	$C_{19}H_{21}N_2O_2S$	–
DP-1	373.1214	373.1222	−2.1	$C_{19}H_{21}N_2O_4S$	+2O
DP-2	375.1368	375.1379	−2.9	$C_{19}H_{23}N_2O_4S$	+2O, +2H
DP-3	357.1269	357.1273	−1.1	$C_{19}H_{21}N_2O_3S$	+O
DP-4	357.1266	357.1273	−2.0	$C_{19}H_{21}N_2O_3S$	+O

[a] Difference in molecular formula between Compound A and degradation products.

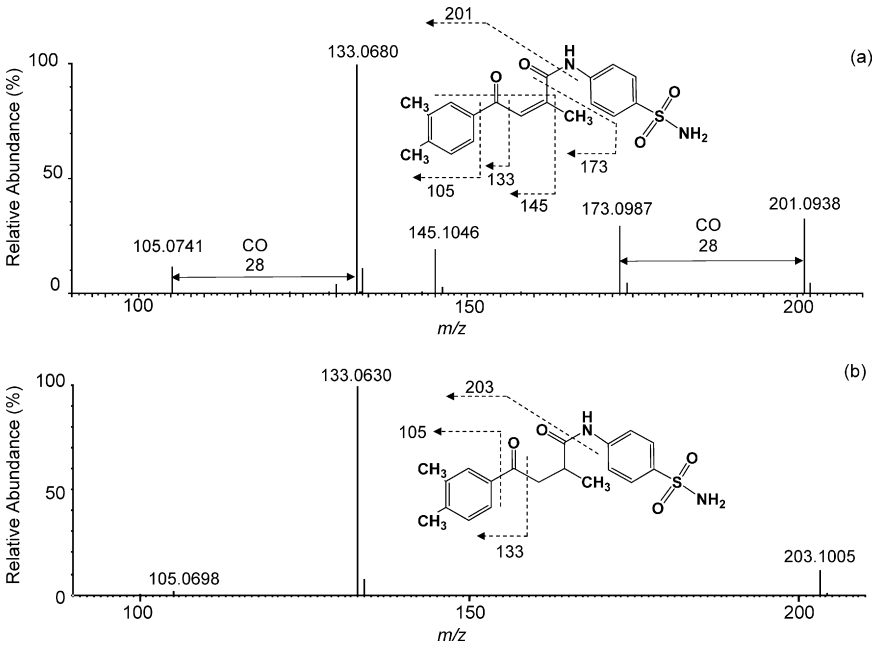

Fig. 3. LC–MS/MS spectra of in-source fragment ions of (a) DP-1 at *m*/*z* 201 and (b) DP-2 at *m*/*z* 203.

preparation of the mobile phases. A 4–60 μL aliquot of the sample solution was subjected to analysis. For the NMR experiments, a ^{1}H–^{13}C/^{15}N triple-resonance inverse (TCI) 5 mm cryoprobe (active volume: 60 μL) with cold ^{1}H- and ^{13}C-coils, preamplifiers and a *z*-gradient accessory was used. ^{1}H NMR spectra were recorded using the double presaturation nuclear Overhauser effect spectroscopy pulse sequence with shaped pulses for the suppression of acetonitrile and water signals. Spectra were acquired with a 12019 Hz spectral width and 16 K data points, giving a digital resolution of 0.73 Hz per point. A total of 512–1024 scans were accumulated. All the spectra were processed with an exponential function, a line broadening of 1 Hz and a zero filling factor of 2. All the NMR spectra were recorded at a constant temperature of 25 °C and the chemical shifts were referenced to the methyl signal of the residual acetonitrile at 1.93 ppm.

3. Results and discussion

3.1. Stability study of Compound A

3.1.1. Stability study of Compound A

During the stability studies of the Compound A drug substance and its tablets under high humidity and thermal conditions, an increase in four degradation products, DP-1, DP-2, DP-3 and DP-4 was observed. The chromatograms are shown in Fig. 2. After storage of the drug substance for 16 weeks at 40 °C/75% RH, the

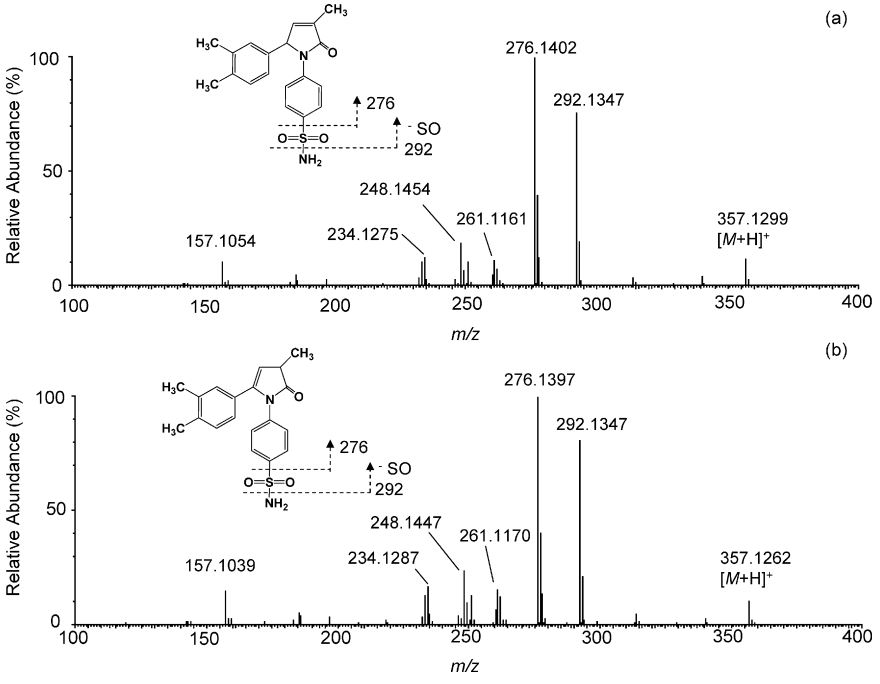

Fig. 4. LC–MS/MS spectra of (a) DP-3 and (b) DP-4.

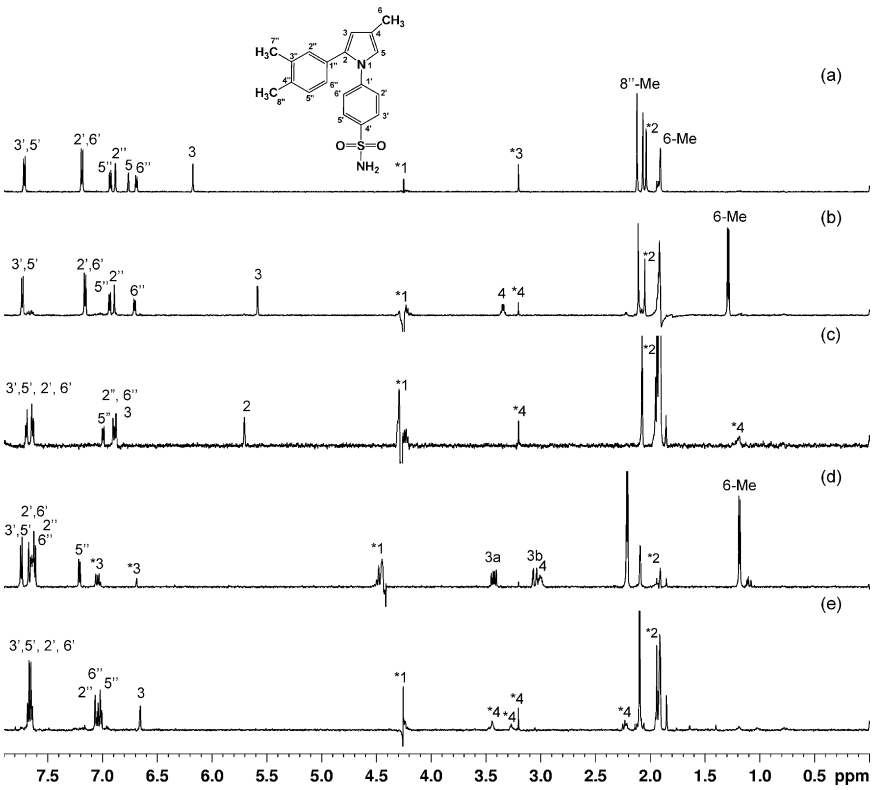

Fig. 5. LC-^{1}H NMR spectra of (a) Compound A, (b) DP-4, (c) DP-3 (d) DP-2 and (e) DP-1. Asterisks denote the signals of water (*1), acetonitrile (*2), contamination of DP-1 (*3) and impurities (*4) in the mobile phase.

levels of DP-1, DP-2, DP-3 and DP-4 were 1.12%, 0.33%, 0.21% and 0.28%, respectively. The corresponding values for the storage conditions at 60 °C were 1.91%, 0.17%, 0.35% and 0.32%. In the case of the tablets, after storage for 4 weeks at 40 °C/75% RH, the same degradation products were observed at 4.95%, 0.08%, 1.37% and 0.28%, respectively. Under storage conditions at 60 °C, the same three degradation products, DP-1, DP-3 and DP-4 were observed at 1.56%, 0.68% and 0.34%, respectively, and DP-2 was less than 0.01%. In order to elucidate the degradation mechanism, the chemical structures of DP-1, DP-2, DP-3 and DP-4 were determined.

3.1.2. Identification of degradation products and degradation pathway

LC–MS and LC-^{1}H NMR were used to determine the chemical structures of the degradation products [21–27]. The molecular formulas obtained from the accurate mass results are listed in Table 1, and the MS and ^{1}H NMR spectra are shown in Figs. 3–5.

Regarding DP-1 and DP-2, in-source fragment ions corresponding to the elimination of the 4-sulfamoylphenyl amino moiety were observed at *m*/*z* 201 and *m*/*z* 203 in their MS spectra respectively and the product ions in the MS/MS spectra indicated that DP-1 and DP-2 both have a pyrrole ring-opened structure by the generation

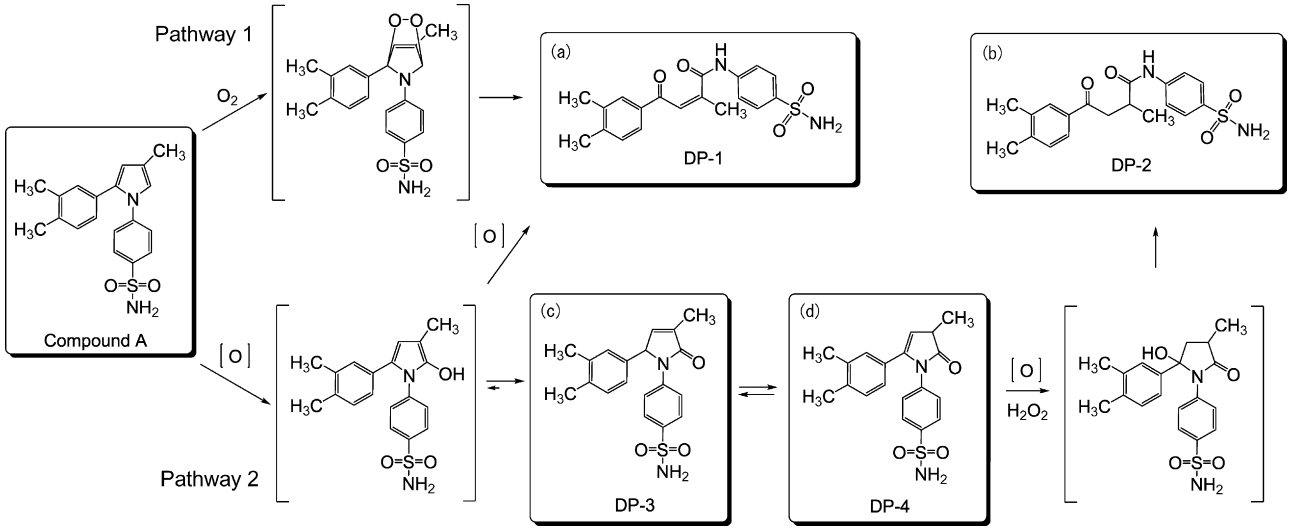

Fig. 6. Proposed degradation pathway of Compound A.

Table 2
Comparison of oxidative degradation products in Compound A stored under moisture-saturated conditions using various oxidative media for 1 day.

				Content (%)
Oxidative media	Control [a]	H_2O	3% H_2O_2	1 mM RB[b]
Temperature	60 °C	60 °C	60 °C	40 °C
Degradation product				
DP-1	0.21	0.64	2.31	0.64
DP-2	<0.01	0.09	0.98	0.05
DP-3	0.07	0.13	0.38	0.12
DP-4	0.15	0.16	0.32	0.25

[a] Ambient air.
[b] Rose bengal.

of two carbonyl groups. These structures were also confirmed by their ^{1}H NMR spectra. As for DP-2, the saturation of the C3–C4 bond was also indicated by observation of the C4 methine proton and the chemical shifts of C6 methyl protons and C3 methylene protons in the ^{1}H NMR spectrum.

DP-3 and DP-4 are considered to be isomers since similar fragmentation patterns related to aromatic sulfonates were observed in their MS/MS spectra [28,29], and to be a carbonyl form at the C5 position of the pyrrole ring, since no proton signals of C5 were observed in their ^{1}H NMR spectra. Moreover, a methine proton signal of C2 for DP-3 and one of C4 for DP-4 were observed in their respective spectra and it was noted that double bonds exist at C3–C4 in DP-3 and at C2–C3 in DP-4 [30], indicating that they are tautomers.

The four degradation products detected in the stability studies were identified. The degradation pathway was established from the chemical structures of these degradation products, as shown in Fig. 6 [31–34], and the degradation products were all determined to be generated by oxidation of the pyrrole ring. The proposed pathways are Pathway 1, in which the pyrrole ring opens due to the reaction of oxygen with C2 and C5 of the pyrrole ring, leading to the generation of DP-1; and Pathway 2, in which carbonyl groups are formed by the reaction of oxygen on the C5 position of the pyrrole ring, with the double bond transferring, leading to isomerization and the generation of DP-3 and DP-4. In Pathway 2, it is thought that 5-hydroxylated Compound A, a reactive intermediate, and DP-4 were further oxidized, thus leading to the generation of DP-1 and DP-2 after opening of their pyrrole rings, respectively. From these results, the use of oxygen and/or oxidizing agents was considered to be effective in establishing experimental systems for the prediction of these oxidative degradation products.

3.2. Development of stress conditions

In order to develop a prediction system for the degradation of Compound A, effective oxidative stress conditions were investigated based on the proposed degradation pathway. Solid-state Compound A was stored under moisture-saturated conditions using aqueous solutions to which various oxidizing agents had been added. The effects of oxygen in the headspace were also investigated.

3.2.1. Selection of oxidative media

Water, hydrogen peroxide and rose bengal solutions were investigated as solutions used to saturate the headspace with moisture. Hydrogen peroxide is widely used as an oxidizing agent and rose bengal is known to generate singlet oxygen from oxygen molecules in a ground state upon photoexcitation. After storage at 60 °C or 40 °C, the levels of the oxidative degradation products generated were measured. A sample stored in ambient air without saturated moisture was used as the control. In the system using rose bengal solution, the generation of singlet oxygen was promoted by irradiating the solution with light at 2500 lux.

By storing solid-state Compound A under moisture-saturate conditions, the degradation products were increased compared to the control and significant increase was observed in the system using hydrogen peroxide, as shown in Table 2. After storage for 1 day at 60 °C in the hydrogen peroxide system, the degradation products detected were DP-1, DP-2, DP-3 and DP-4 and the levels were 2.31%, 0.98%, 0.38% and 0.32%, respectively. In the system with rose bengal solution at 40 °C, the degradation product profile obtained was similar to that in the system using water at a higher storage temperature of 60 °C. For further investigation, the rose bengal concentration was varied from 0.1 mmol/L to 100 mmol/L and oxygen was also substituted for the ambient air in the headspace, but no remarkable change in the degradation product profile was observed.

From these results, it was indicated that degradation of Compound A can occur within a short period of time by storage under moisture-saturated conditions and that its degradation can be further accelerated by hydrogen peroxide. Moreover, the same degradation products that were observed in the stability studies were detected and it was possible to predict the potential degradation products within a short time by using the proposed experimental systems.

On the other hand, the experimental system using the photosensitizer rose bengal in order to investigate oxidation by singlet oxygen did not indicate significant influence in this study. However, the degradation of pyrrole by singlet oxygen has been reported [31,35] and thus further optimization of the experimental conditions, as well as further study of other photosensitizers, is considered to be necessary.

3.2.2. Effect of oxygen in headspace

The effect of headspace oxygen on degradation in the prediction system was investigated. Hydrogen peroxide moisture-saturated conditions were used and oxygen and nitrogen were substituted for the ambient air in the headspace. After storage for 1 day at 60 °C, the levels of DP-1, DP-2, DP-3 and DP-4 in the oxygen headspace system were 4.19%, 1.54%, 0.56% and 0.25%, respectively, compared to levels of 1.25%, 0.55%, 0.29% and 0.25% for the nitrogen headspace system. Thus, although no significant difference in the levels of DP-4 was observed between the oxygen and nitrogen headspace systems, the levels of DP-1, DP-2, and DP-3 increased significantly in the oxygen headspace system. This result is consistent with the proposed degradation pathway shown in Fig. 6. Regarding DP-4, no significant change was observed due to acceleration of the oxidation reaction to DP-2 by the increase in the amount of oxygen in the headspace.

These results demonstrated that the substitution of headspace air in the prediction system with oxygen is effective for predicting oxidative degradation.

3.2.3. Optimization of hydrogen peroxide moisture-saturated conditions

The system using hydrogen peroxide moisture-saturated conditions with oxygen was found to be useful for predicting oxidative

Table 3
Comparison of oxidative degradation products in Compound A stored under various concentrations of hydroxyl peroxide moisture-saturated conditions with oxygen at 40 °C for 1 day.

				Content (%)
Degradation product	H_2O_2 concentration (%)			
	Control[a]	0.3	3.0	30
DP-1	0.50	0.53	0.66	2.96
DP-2	0.08	0.10	0.39	0.50
DP-3	0.12	0.13	0.18	1.00
DP-4	0.19	0.21	0.18	0.28

[a] Water.

degradation. In forced degradation studies, however, the conditions are often overly severe for the prediction of the degradation products that would increase under actual storage conditions, and/or sometimes lead to unnecessary secondary degradation. Therefore, the hydrogen peroxide concentration and storage temperature were optimized.

3.2.3.1. Effect of hydrogen peroxide concentration. The hydrogen peroxide concentration was varied at concentrations of 0.3%, 3.0%, and 30%, and the levels of oxidative degradation products generated after storage at 40 °C were investigated. Oxygen was substituted for the ambient air in the headspace and a system utilizing water was used as a control. As shown in Table 3, no significant difference in the levels of the oxidative degradation products was observed in the system using 0.3% hydrogen peroxide compared to the control, which indicated that hydrogen peroxide had had no effect at that concentration. On the other hand, in the systems using 3% and 30% hydrogen peroxide, the levels of the oxidative degradation products were significantly increased. After storage for 1 day in the system using 3% hydrogen peroxide, the levels of DP-1, DP-2, DP-3 and DP-4 were 0.66%, 0.39%, 0.18%, and 0.18%, respectively. In the system using 30% hydrogen peroxide, they were 2.96%, 0.50%, 1.00% and 0.28%, respectively. However, in the system using 30% hydrogen peroxide, degradation products that were not detected under actual storage conditions were observed, as the chromatogram in Fig. 7 shows.

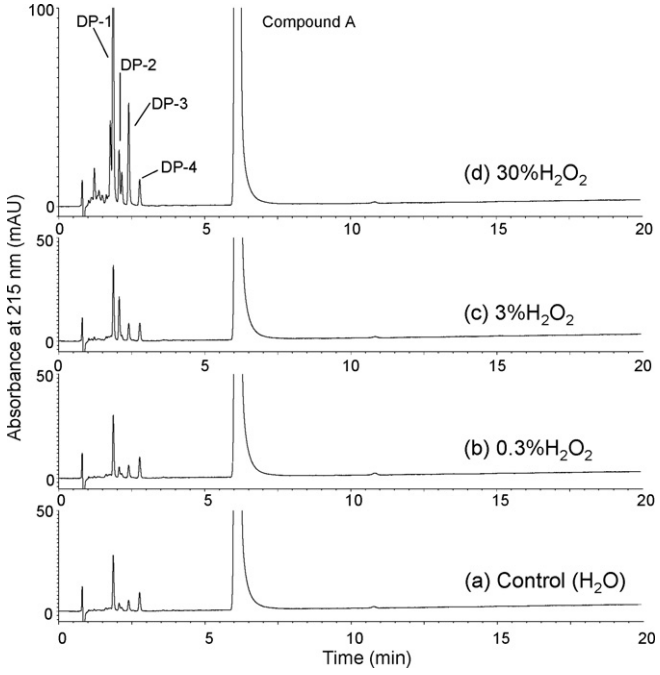

Fig. 7. Chromatograms of Compound A stored under various concentrations of hydroxyl peroxide moisture-saturated conditions with oxygen at 40 °C for 1 day.

Table 4
Content of oxidative degradation products in Compound A after storage at 25, 40 and 60 °C under 3% hydrogen peroxide moisture-saturated conditions with oxygen.

					Content (%)
Temperature (°C)	Degradation product	Storage period (day)			
		0	1	3	5
25	DP-1	0.14	0.30	0.38	0.44
	DP-2	<0.01	0.08	0.15	0.20
	DP-3	0.04	0.10	0.12	0.12
	DP-4	0.12	0.21	0.22	0.16
	Total of DPs[a]	0.30	0.69	0.87	0.92
40	DP-1	0.14	0.66	1.41	1.63
	DP-2	<0.01	0.39	1.13	1.53
	DP-3	0.04	0.18	0.35	0.42
	DP-4	0.12	0.18	0.26	0.23
	Total of DPs[a]	0.30	1.41	3.15	3.81
60	DP-1	0.14	4.19	–	–
	DP-2	<0.01	1.54	–	–
	DP-3	0.04	0.56	–	–
	DP-4	0.12	0.25	–	–
	Total of DPs[a]	0.30	6.54	–	–

(–) Not evaluated due to excessive degradation.
[a] Degradation products.

In the system using 3% hydrogen peroxide, on the other hand, a degradation product profile similar to that actually observed in the stability studies was observed, as shown in the chromatogram in Fig. 7.

From these results, 3% was considered to be an appropriate concentration of hydrogen peroxide to predict the oxidation of Compound A.

3.2.3.2. Effect of temperature. The storage temperature was varied at temperatures of 25 °C, 40 °C and 60 °C to investigate the effect of temperature on the degradation of Compound A. The hydrogen peroxide concentration used was 3% and the oxygen headspace system was used. The levels of each oxidative degradation product are presented in Table 4 and the chromatograms are shown in Fig. 8

The levels of DP-1, DP-2, DP-3 and DP-4 generated after 1 day of storage at 25 °C were 0.30%, 0.08%, 0.10% and 0.21% for, respectively. The longer the storage period the greater the increase of DP-1 and DP-2, but the levels of DP-3 and DP-4 did not increase even after

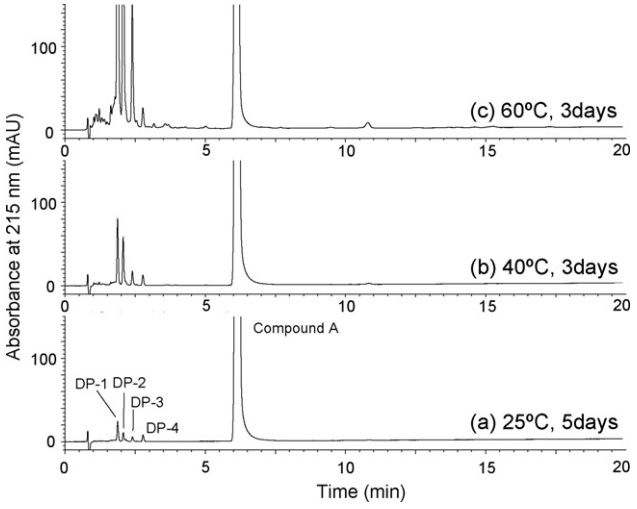

Fig. 8. Chromatograms of Compound A after storage under 3% hydrogen peroxide moisture-saturated conditions with oxygen at (a) 25 °C for 5 days, (b) 40 °C for 3 days and (c) 60 °C for 3 days.

storage for 5 days. The levels of DP-1, DP-2, DP-3 and DP-4 generated after 5 days of storage were 0.44%, 0.20%, 0.12% and 0.16%, respectively. Although similar degradation product profiles were obtained at 25 °C as for the stability studies under actual storage conditions, the rate of the degradation product formation was low and some degradation products did not increase dependent upon the storage period, as shown in the chromatograms in Fig. 8.

Storage at 40 °C for 1 day was also performed, and DP-1, DP-2, DP-3 and DP-4 were detected at 0.66%, 0.39%, 0.18% and 0.18%, respectively. These oxidative degradation products increased further after storage for 3 days and the levels were 1.41%, 1.13%, 0.35% and 0.26%, respectively. As shown in Fig. 8, the chromatograms obtained were similar to the profiles obtained in the stability studies. With storage for 5 days, an even greater increase could be observed, yielding sufficient amounts of the degradation products to obtain information on their chemical structures by using hyphenated techniques such as LC–MS, LC-NMR, etc.

The most dramatic increase in oxidative degradation product levels was obtained at 60 °C. After 1 day of storage, DP-1, DP-2, DP-3 and DP-4 increased to 4.19%, 1.54%, 0.56% and 0.25%, respectively. These levels increased over time during the storage period, but as shown in the chromatogram in Fig. 8, degradation products that were not actually observed in the stability studies were also detected. On the basis of these observations, a temperature condition of 60 °C was not considered to be appropriate for predicting possible degradation products.

As described above, a condition of 25 °C is too mild for the prediction of all of the oxidative degradation products in the short term. A condition of 60 °C, on the other hand, was too severe. On the basis of these results, storage conditions of 40 °C and a 3-day storage period were considered to be optimal for the prediction of oxidative degradation in the short term. From the degradation product profile obtained, it is considered that the same degradation reaction as in actual storage of the solid-state Compound A had occurred. Therefore, this developed prediction system is considered to be useful for predicting oxidative degradation products that could be generated under actual storage conditions.

4. Conclusions

Stress conditions for predicting possible oxidative degradation products in solid-state pharmaceutical compounds were investigated using Compound A as a model compound. The four main degradation products of Compound A were identified as oxidation products and then a prediction system for the oxidation reaction was developed. The solid-state Compound A was stored under moisture-saturated conditions using aqueous solution with hydrogen peroxide as the oxidant and oxidative degradation occurred within a short period of time. Moreover, substitution of the headspace air in the prediction system with oxygen was effective in accelerating the degradation even further. After optimization of the conditions, a similar degradation product profile to that actually observed in stability studies was successfully obtained in only 3 days under conditions using 3% hydrogen peroxide at 40 °C. The prediction system developed in this study was effective for the prediction of the oxidative degradation of solid-state Compound A. Since oxidation is a commonly occurring degradation mechanism in pharmaceuticals, the developed prediction system would be applicable to other pharmaceuticals. The information regarding degradation product profiles and pharmaceutical stability obtained using this newly developed prediction system will be useful for the development of stable pharmaceutical formulations and the selection of suitable packaging configurations at the early stage of drug development.

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