Elucidation of Degradation Behavior of Nitrazepam and Other Benzodiazepines in Artificial Gastric Juice: Study on Degradability of Drugs in Stomach (II)

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The degradation behavior of eight benzodiazepines (BZPs): alprazolam, etizolam, diazepam, triazolam, nitrazepam (NZP), flunitrazepam (FNZ), bromazepam, and lorazepam, in artificial gastric juice was monitored by a LC/photodiode array detector (PDA) to estimate their pharmacokinetics in the stomach. For drugs that were degradable, such physicochemical parameters as reaction rate constant were measured to evaluate the effect of storage conditions on drug degradability, such as whether the degradation proceeds faster by increasing storage temperature, or whether the degradation reaction is reversible by adjusting pH. As a result, it was found that although the eight BZPs degraded in artificial gastric juice, most of them could be restored when pH was increased, and the restoration rates differed depending on the pH and the type of BZR. As for NZP, an Arrhenius plot was drawn to obtain the physicochemical parameters, such as activation energy and activation entropy involved in the degradation reaction, and the reaction kinetics was discussed. In addition, two substances were confirmed as the degradation products of NZP in artificial gastric juice: one was a reversible degradation product (A) (intermediate) and the other was an irreversible degradation product (B) (final degradation product). The intermediate was identified as 2-amino-N-(2-benzoyl-4-nitrophenyl)-acetamide, and the final degradation product was 2-amino-5-nitrobenzophenone. Therefore, when detecting NZP in human stomach contents, such as during judicial dissection, it would be prudent to target NZP as well as the intermediate (A) and the final degradation product (B).

Key words benzodiazepine; nitrazepam; degradation pathway; artificial gastric juice; HPLC

Introduction

Benzodiazepines (BZPs) are frequently used as an anti-convulsant and anxiolytic in addition to a sedative.1 However, BZPs have been sometimes used in such criminal acts as robbery, murder, and rape after a woman lapses into a coma.2 In cases where such drug use is suspected, drug appraisal is often performed after some time has passed since the crime, and it may be difficult to detect drugs that do not have long-term stability in the body.3 In fact, in one murder case in which flunitrazepam (FNZ) use was suspected,4 it was difficult to detect the suspected drug from the biological sample of the victim. As one of the reasons, it was presumed that FNZ was degraded due to long-term exposure to gastric acidity. Unfortunately, pharmacokinetic studies of therapeutic sleeping pills do not assume long-term storage in gastric juice and thus, there is insufficient evidence to indicate that FNZ is degraded in gastric juice. Studies on drug stability have been conducted, but these are mainly studies5–7 on the stability in blood and urine of such psychotropic drugs as cocaine, morphine, and Lysergsäurediethylamid (LSD). The stability of BZPs has been reported in blood and plasma,8,9 as well as bile and vitreous humor of postmortem samples.10 With regard to the physical properties of BZPs, it is known that the azomethine bond of the diazepine ring in many BZPs is cleaved off under acidic conditions, although ring closure occurs under neutral conditions.10,11 These studies suggest that orally ingested BZPs are degraded in the stomach but restored to their original state in the small intestine. In addition, in the experiments based on these reports, the acidic condition for degradability verification is simply created by using dilute hydrochloric acid, and there are almost no data from in vivo research. Similarly, in the restoration experiments (ring closure reactions), little has been said about the effects of pH.

Although it is presumed that this degradation reaction (ring-opening reaction) proceeds via an intermediate, reports of such are few. This is because BZR intermediates are unstable and difficult to isolate and purify. For this reason, past studies of BZR degradation behavior inferred the time course of the degradation by monitoring the acid hydrolysis of BZPs by spectrophotometry10,13 and polarography.12

In our previous work,13 we elucidated the degradation behavior of tricyclic antidepressant amoxapine in artificial gastric juice. In this study, we reconfirmed the degradation behavior of eight frequently used BZPs (alprazolam, etizolam, diazepam, triazolam, nitrazepam (NZP), FNZ, bromazepam, and lorazepam) in artificial gastric juice to infer their pharmacokinetics in stomach. We also investigated the restoration of BZPs to their original state by varying the pH of the solution from weakly acidic to basic, in order to clarify the labile behavior if pretreatment of gastric contents and sample is not carried out properly during the appraisal work for BZPs. The behavior was monitored by using a LC/photodiode array detector (PDA). LC/PDA was able to separate BZPs and their degradation products, as well as measure their UV spectra for identification.

As for NZP, it was reported that 2-amino-5-nitrobenzophenone was actually isolated and purified as the degradation product under acidic conditions.12,14 On the other hand,
although the existence and the structural formula of an intermediate were estimated, its detailed structural information could not be determined because the intermediate was difficult to isolate and purify. Only Davidson and Smail were not able to isolate the acid hydrolysate of NZP (intermediate and final degradation product) by liquid–liquid extraction and to estimate the intermediate by instrumental analyses. Although the intermediate was reported to be 2-glycylamino-5-nitrobenzophenone (as was already predicted by Han et al.), raw spectral data were not sufficiently provided and no detailed discussion was made on the degradation behavior of NZP into the intermediate and further of the intermediate into the final degradation product.

Therefore, in this study, we tried to isolate and purify the intermediate and the final degradation product of NZP using solid-phase extraction, and performed structural analysis using LC/time-of-flight (TOF)-MS and NMR spectroscopy. For NZP and its intermediate, we examined such physico-chemical parameters as reaction rate constant (k), activation energy (Ea), and activation entropy (AS) under acidic conditions using artificial gastric juice to mimic the acidic state in the stomach, and discussed the degradation behavior of NZP.

**Experimental**

**Materials**

Alprazolam, etizolam, diazepam, triazolam, NZP, FNZ, bromazepam, and lorazepam (all biochemical grade) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). The chemical structures of the eight BZPs are shown in Fig. S1. Each standard was dissolved in methanol to make a 10 mg/mL standard stock solution. Working standard solutions were then prepared from each standard stock solution by dilution with methanol.

Acetonitrile and methanol (both HPLC grade); formic acid, phosphoric acid, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium tetraborate, 10-hydrate (borax; all special grade) sodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium tetraborate, 10-hydrate (borax; all special grade) were from Kanto Chemical Co., Inc. (Tokyo, Japan). Water was purified with a Milli-Q® polisher (Merck Ltd., Darmstadt, Germany). All other chemicals were of special grade.

An Oasis® HLB 6cc Vac Cartridge (150 mg, particle size 30 µm; Waters Co., Milford, MA, U.S.A.) was used. The cartridge was conditioned with methanol, water, and 0.05 mol/L HCl prior to use.

The artificial gastric juice and each pH buffer solution were prepared as follows.

Artificial gastric juice (pH 1.2): 2.0 g of sodium chloride was dissolved in 7.0 mL of hydrochloric acid and purified water was added to make 1000 mL; pH 1.2.

Buffer solution (pH 5) was prepared by adding 1 mol/L disodium hydrogen phosphate aqueous solution to 1 mol/L sodium dihydrogen phosphate aqueous solution until the pH reached 5.0. Buffer solution (pH 7) was prepared by adding 1 mol/L disodium hydrogen phosphate aqueous solution to 1 mol/L sodium dihydrogen phosphate aqueous solution until the pH reached 7.0. Buffer solution (pH 9) was prepared by adding 1 mol/L sodium dihydrogen phosphate aqueous solution to 0.1 mol/L sodium tetraborate aqueous solution until the pH reached 9.0.

**Apparatus and Operating Conditions**

**LC/PDA**

An L-6300 Intelligent pump (Hitachi, Tokyo, Japan) system equipped with an MD-910 photodiode array detector (PDA; JASCO Corporation, Tokyo, Japan) was used. LC separation was performed with a Poroshell 120 EC-C18 column (100 × 4.6 mm I.D., 2.7 µm; Agilent Technologies, Santa Clara, CA, U.S.A.). Column temperature was maintained at 40 °C. The mobile phase was a mixture of 50 mmol/L potassium phosphate buffer solution (pH 3) and acetonitrile in the ratio of 70:30 or 60:40 (v/v), and was delivered in the isocratic elution mode at the flow rate of 0.5 mL/min. The UV monitoring wavelength was set at 220 nm. A 10 µL aliquot of the sample was injected into the system.

**LC/TOF-MS**

An Alliance HT 2795 HPLC system equipped with an LCT Premier XE TOF-MS (Waters Corporation) was used. LC separation was performed with a Poroshell 120 EC-C18 column (100 × 4.6 mm I.D., 2.7 µm; Agilent Technologies). Column temperature was maintained at 40 °C. The mobile phase was a mixture of 10 mmol/L formic acid aqueous solution and acetonitrile in the ratio of 60:40 (v/v), and was delivered in the isocratic elution mode at the flow rate of 0.5 mL/min. A 10 µL aliquot of the sample was injected into the system.

The optimum working parameters for TOF-MS were as follows: electrospray ionization (ESI): positive mode, capillary voltage: 2000 V, cone voltage: 50 V, aperture #1 voltage: 15 V, desolvation temperature: 350 °C, source temperature: 120 °C, desolvation gas flow (N2): 800 L/h, and cone gas flow (N2): 50 L/h. Leucine-enkephalin solution (0.25 µg/mL) was delivered at the flow rate of 5 µL/min. Mass accuracy was maintained using Lock-Spray with the leucine-enkephalin [M + H]⁺ ion, m/z = 556.2771, as the lock mass. The resolution was at least 10000 as calculated by using the full width at half-maximum method.

**NMR**

An ECA-600II NMR spectrometer (JEOL, Tokyo, Japan) was used as the NMR apparatus. The frequency for ¹H-NMR measurement was set at 600.1723 MHz, and that for ¹³C-NMR measurement was set at 150.9134 MHz. As the reference peak, solvent peaks of methanol-d₄ (¹H: 3.31 ppm, ¹³C: 49.0 ppm) were used.

As the other apparatuses, an SCI-165 CO₂ incubator (ASTEC, Tokyo, Japan) and an MOV-112S dry heat sterilizer (SANYO, Tokyo, Japan) were used as the storage device at 38 to 70 °C.

**Degradation Behavior of BZPs in Artificial Gastric Juice**

Degradation Experiment

To 900 µL of artificial gastric juice was added 100 µL of each BZP standard solution (1000 µg/mL, alprazolam, etizolam, diazepam, triazolam, NZP, FNZ, bromazepam, and lorazepam). Then, the solutions were stored in an incubator set to 38 °C and time-dependent degradation over a 24 h period (0.5, 2, 4, 6, and 24 h) was determined by LC/PDA measurements. In the accelerated degradation test for NZP, which was confirmed to be degraded in the degradation experiment, NZP was dissolved in artificial gastric juice similar to the above and stored at 5, 25, 50, 60, and 80 °C. The time course of NZP degradation was monitored by LC/PDA. The LC/PDA system equipped with an MD-910 photodiode array detector (PDA; JASCO Corporation, Tokyo, Japan) was used. Column separation was performed with a Poroshell 120 EC-C18 column (100 × 4.6 mm I.D., 2.7 µm; Agilent Technologies). Column temperature was maintained at 40 °C. The mobile phase was a mixture of 50 mmol/L potassium phosphate buffer solution (pH 3) and acetonitrile in the ratio of 70:30 or 60:40 (v/v), and was delivered in the isocratic elution mode at the flow rate of 0.5 mL/min. The UV monitoring wavelength was set at 220 nm. A 10 µL aliquot of the sample was injected into the system.
degradation was determined by LC/PDA measurements.

Restoration Experiment of BZPs
In experiments to verify the restoration behavior of BZPs from their degradation products by pH readjustment, three kinds buffer solutions adjusted to pH 5, 7, and 9 were used. To 900 µL of each buffer solution, 100 µL of each BZP degradation solution, which was preliminarily prepared by adding 100 µL of BZP standard stock solution (10 mg/mL) to artificial gastric juice (pH 1.2) and storing at 38 °C for 24 h, was added. Then, the solutions were stored at room temperature (25 °C) and time-dependent changes over an approximately 2 h period (0, 0.5, 1, and 2 h) were determined by LC/PDA measurements.

Isolation and Purification of NZP Degradation Products
Reversible Degradation Product (A)
Thirty milligrams of NZP was dissolved in 30 mL of artificial gastric juice and the solution was stored at 50 °C for 24 h. The solution was purified using the solid-phase extraction cartridge Oasis® HLB cartridge (150 mg × 6). Five milliliters of the degradation solution described above was processed in one Oasis® HLB cartridge. After loading the supernatant and washing with 5 mL of 0.05 mol/L HCl aqueous solution, 5 mL of 40% methanol/0.05 mol/L HCl aqueous solution was used for elution from the solid phase. All the eluted solutions were combined, 30 mL of 100% ethanol was added, and azeotropic distillation was carried out with an evaporator. The residue was redissolved in 100% methanol, transferred into a microtube, and dried by nitrogen purge to isolate reversible degradation product (A) as a yellow powder.

Results and Discussion
Operating Conditions for LC/PDA
LC/PDA measurement conditions were examined with reference to published papers.13,16 Figure 1 shows the representative chromatogram of the standard solutions (20 µg/mL) of the eight BZPs. The eight BZPs were sufficiently separated by isocratic elution. The limit of detection (LOD) (S/N = 3) and the limit of quantification (LOQ) (S/N > 10) of the BZPs were 0.5–1.0 and 2.0–5.0 µg/mL, respectively. The calibration curve showed good linearity (r > 0.999) in the range of each LOQ to 100 µg/mL.

Degradation Verification in Artificial Gastric Juice
In order to verify the degradability of BZPs in artificial gastric juice, the degradation behavior of BZPs in artificial gastric juice (pH 1.2) was observed over 24 h, and it was found that the time course could be classified into three patterns depending on the type of drug: (I) alprazolam, bromazepam, triazolam, and FNZ degraded rapidly within 30 min in artificial gastric juice and the degradation plateaued thereafter; (II) diazepam and etizolam gradually degraded with time until approximately 6 h later and the degradation plateaued thereafter; and (III) NZP and lorazepam tended to degrade gradually over 24 h (Fig. 2). BZPs concentrations remaining after 24 h were as follows: alprazolam (7.85 µg/mL), bromazepam (11.8 µg/mL), triazolam (19.5 µg/mL), FNZ (48.6 µg/mL), diazepam (66.3 µg/mL), etizolam (32.5 µg/mL), NZP (7.70 µg/mL), and lorazepam (42.0 µg/mL).

NZP Long-Term Degradability Verification Experiment
NZP is known to form benzophenone compounds as the final degradation products through intermediates under acidic conditions.10–13 From a detailed re-examination of the degradability of NZP in artificial gastric juice, we confirmed that two degradation products were produced from NZP in addition to NZP itself (Fig. 3). The two peaks (A and B) represented degradation products that had a shorter and a longer retention
time than NZP, respectively. The peak having a short retention time (reversible degradation product (A)) was assigned to the intermediate, and the peak having a long retention time (irreversible degradation product (B)) was assigned to a benzophenone compound as the final degradation product. The fact that irreversible degradation product (B) is 2-amino-5-nitrobenzophenone was confirmed by matching its retention time and UV spectra with those of 2-amino-5-nitrobenzophenone standard by LC/PDA measurement.

**Experiments on Restoration of BZPs by pH Readjustment** A restoration experiment was conducted to confirm whether BZPs could be restored to their original state by changing the pH from acidic to neutral or basic. As a result, we found that the restoration rate could be classified into three patterns depending on the type of drug (Fig. 4), as follows: (I) 90–100% restoration in approximately two hours (alprazolam, etizolam, and triazolam); (II) 60–80% restoration (diazepam, nitrazepam, NZP, FNZ, and bromazepam); and (III) no restoration (lorazepam, alprazolam, etizolam, and triazolam). These compounds have a triazole ring in common, and all of them showed 90–100% restoration by changing the pH in the liquid reconditioning experiment. Previous reports indicated that when BZPs are stored under acidic conditions, the parent compound is degraded into the benzophenone form via the intermediate, but the degradation of the parent compound into the intermediate is reversible. Based on these findings, it was considered that the degradation reaction of alprazolam, etizolam, and triazolam proceeds to yield their respective intermediates in artificial gastric juice. It was speculated that the presence of the triazole ring might have suppressed the degradation from the intermediate into the benzophenone form. In fact, there was a report that hydrolysis was suppressed by the presence of the triazole ring in the structure.

Regarding lorazepam, no quantitative restoration to its original state was observed even if the pH was adjusted from neutral to basic. From this result, it was speculated that the degradation intermediate of lorazepam in artificial gastric juice rapidly changed to the benzophenone form and thus the restoration was not apparent, or the degradation reaction into the intermediate might be poorly reversible. It was also inferred that the hydroxy group in the diazepine ring of lorazepam might have promoted the degradation reaction or hindered the restoration.

From the degradability and restoration experiments, it was speculated that if intermediates were detected instead of parent compounds at the time of judicial dissection, most of the parent compounds would be detected by changing the pH from strongly acidic to basic.

**Structural Analysis of NZP Reversible Degradation Product (A)** NZP reversible degradation product (A) was isolated and purified as a yellow powder (26.2 mg; yield: 87.3%). The reason why dilute hydrochloric acid was allowed to coexist in the washing and elution step of the solid-phase extraction in the purification operation was to prevent the intermediate from reverting to NZP.

UV spectroscopy, MS, and NMR spectroscopy ([1H-NMR, 13C-NMR, 1H-13C correlation spectroscopy (COSY), heteronuclear multiple-bond connectivity (HMBC) correlations, and nuclear Overhauser enhancement and exchange spectroscopy (NOESY)) were carried out. The UV spectra of NZP, reversible degradation product (A), and irreversible degradation product (B) were measured by using LC/PDA. NZP showed UV \( \lambda_{\text{max}} \) peaks at 219, 259, and 311 nm (Fig. 5(I)), whereas reversible degradation product (A) showed UV \( \lambda_{\text{max}} \) peaks at 219, 267, and 311 nm (Fig. 5(II)). In the case of reversible degradation product (A), the peak at 267 nm was large, but because the positions of the UV \( \lambda_{\text{max}} \) peaks for both NZP and reversible degradation product (A) were almost the same, the basic skeleton of reversible degradation product (A), namely, the resonance structure derived from the double bond, was inferred to be similar to NZP. On the other hand, irreversible degradation product (B), 2-amino-5-nitrobenzophenone, had UV \( \lambda_{\text{max}} \) peaks at 239 and 363 nm (Fig. 5(III)), and its spectral pattern was entirely different from those of NZP and reversible degradation product (A).

From the high-resolution MS of reversible degradation product (A) measured by LC/TOF-MS, the protonated molecule \([M + H]^+ m/z 300.604, \text{calcd for } C_{15}H_{13}N_4O_4\) was obtained, and the molecular formula of reversible degradation product (A) was determined to be \( C_{15}H_{13}N_4O_4 \) (Mw: 299.091).

The NMR spectrum of isolated reversible degradation product (A) measured in 600 \( \mu \)L of deuterated methanol (CD3OD) indicated the presence of a monosubstituted benzene ring \( [\delta 7.81 (2H, d, J = 7.81 Hz), \delta 7.68 (1H, t, J = 7.81 Hz), \text{and } \delta 7.55 (2H, t, J = 7.81 Hz)] \), and a 1,2,4-trisubstituted benzene ring \( [\delta 8.45 (1H, d, J = 7.81 Hz), \delta 8.31 (1H, brs), \text{and } \delta 8.22 (1H, d, J = 7.81 Hz)] \), and two protons \( [\delta 3.84 (2H, s)] \) derived from sp3 carbon (Table S1). The number of protons was predicted to be 13 from the high-resolution mass spectrum. Therefore, it was inferred that the three deficient hydrogens were derived from two amino groups.

From the 13C-NMR spectrum of reversible degradation product (A), 14 sp3 carbons, including a carbonyl carbon (\( \delta 196 \) ppm), an amide group carbon (\( \delta 167 \) ppm), and an sp3 carbon (\( \delta 42.6 \) ppm), were assigned as shown in Table S1.
The COSY, HMBC, and NOESY correlations are shown in Table S1 and Fig. 6. As a result of structural analyses, reversible degradation product (A) was confirmed to be 2-amino-N-(2-benzoyl-4-nitrophenyl)-acetamide (Fig. 6). The raw NMR spectra of reversible degradation product (A) are shown in Supplementary Materials (Figs. S2–6).

The structural formula of the intermediate was the same as that inferred in past literature,\(^{10,13,14}\) that is, the 4,5-azomethine bond of the diazepine ring of NZP was broken and glycine was cleaved off. The reason why the 4,5-azomethine bond was broken was considered that the nitro group at the 7-position, which is an electron-withdrawing group, reduced the electron density in A ring, resulting in the cleavage of the conjugated azomethine bond.\(^{14}\)

**Calculation of Physicochemical Parameters in Degradation Reactions of NZP and Intermediate in Artificial**
In order to confirm the degradation pathway of NZP, such physicochemical parameters as reaction rate constant \((k)\), activation energy \((E_a)\), and activation entropy \((\Delta S)\) in the degradation reaction were calculated using the following formulas.

\[
\ln k = -\frac{E_a}{RT} + \ln A
\]

\[
\Delta S = \left(\ln A - \ln \frac{K_B T}{h}\right) R
\]

\(k\): Reaction rate constant, \(A\): Frequency factor, \(R\): Gas constant, \(T\): Absolute temperature, \(K_B\): Boltzmann constant, \(h\): Planck constant

As a result, it was found that the degradation of NZP was a pseudo first-order reaction (Fig. S7). Furthermore, when an Arrhenius plot of the degradation reaction of NZP was drawn (Fig. S8) and its physicochemical parameters were determined, \(E_a\) was 57.9 kJ/mol and \(\Delta S\) was \(-140.4\) J/K·mol.

The degradation reaction of NZP reversible degradation product (A) (intermediate) into irreversible degradation product (B) (final degradation product, benzophenone form) was also analyzed on the basis of reaction kinetics and found to be a pseudo first-order reaction, similar to the degradation reaction of NZP into the intermediate (Fig. S9). Based on the Arrhenius plot, \(E_a\) was 60.14 (kJ/mol) and \(\Delta S\) was \(-169.7\) (J/K·mol) (Fig. S10). Regarding \(E_a\), the value for the degradation reaction of the intermediate into the final degradation product was higher than that for the degradation reaction of NZP into the intermediate. Regarding \(\Delta S\), the value for the degradation reaction of the intermediate into the final degradation product was lower than that for the degradation reaction of NZP into the intermediate. From this, it was shown that the degradation of the intermediate into the final degradation product is less likely to proceed than the degradation of NZP into the intermediate.

**Gastric Juice** In order to confirm the degradation pathway of NZP, such physicochemical parameters as reaction rate constant \((k)\), activation energy \((E_a)\), and activation entropy \((\Delta S)\) in the degradation reaction were calculated using the following formulas.

\[
\ln k = -\frac{E_a}{RT} + \ln A
\]

\[
\Delta S = \left(\ln A - \ln \frac{K_B T}{h}\right) R
\]

\(k\): Reaction rate constant, \(A\): Frequency factor, \(R\): Gas constant, \(T\): Absolute temperature, \(K_B\): Boltzmann constant, \(h\): Planck constant

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the parent compound (NZP) into the intermediate. In the sequential reaction, it was considered that the degradation reaction of the intermediate into the final degradation product was the rate-determining reaction because this slow reaction was the rate-determining step. This is consistent with the experimental finding that NZP was recovered from the intermediate, supporting the above reasoning.

Conclusion

It was confirmed that BZPs are degraded under acidic conditions such as artificial gastric juice, but the degradability differs depending on the type of BZP. On the other hand, it is possible to restore the respective parent compounds from the degraded BZPs when the pH of the solution is changed from acid to neutral or basic. Furthermore, in the restoration process, the restoration rate varies depending on the type of BZP, and is classified into three patterns: (I) 90–100% restoration, (II) 60–80% restoration, and (III) no restoration. We found that NZP was degraded in artificial gastric juice, producing two degradation products, i.e., the intermediate and the final degradation product. The intermediate was 2-amino-N-(2-benzoyl-4-nitrophenyl)-acetamide and the final degradation product was 2-amino-5-nitrobenzophenone. Regarding the degradation and the restoration behavior of NZP, parent compound NZP could be restored from the intermediate, but when the degradation progressed further into the benzophenone form, which is the final degradation product, the reversible reaction of the benzophenone form to yield the intermediate was not possible. Compared with the degradation reaction of NZP into the intermediate, the degradation reaction of the intermediate into the benzophenone form had a high $E_a$ and a low $\Delta S$; thus, it became clear that the latter reaction might be the rate-determining reaction in the NZP degradation reaction. In forensic science or clinical science where the use or abuse of NZP is suspected, when NZP is detected from human stomach contents such as at the time of judicial dissection, it would be prudent to examine both the intermediate and the benzophenone form.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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